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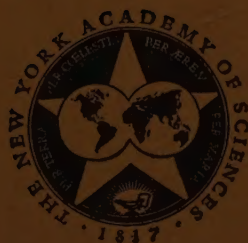
TROPHOBLAST AND ITS TUMORS

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WILLIAM B. OBER

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Part I. Fundamental Concepts

HISTORICAL PERSPECTIVES ON TROPHOBLAST AND ITS TUMORS

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Two separate lines from the poem *Gerontion* by T. S. Eliot provide a fitting introduction to this monograph:

“To lose beauty in terror, terror in inquisition”

and

“History has many cunning passages, contrived corridors
And issues”

I am tempted to apply the first of these passages to the subjective feelings of the microscopist as he examines trophoblastic tissue from a trophoblastic growth. The first impression is one of esthetic pleasure at the arrangement and disposition of cells, their tinctorial reactions, and their relations to the substance and stroma of the organ in which they are situated. The appreciation of histological beauty soon gives way to terror as the gravity of the situation for the patient becomes apparent. Neoplasms of trophoblast are notoriously dangerous and often unpredictable. They usually occur in young women, women of the childbearing age, and the clinical stakes are high. It is this feeling of terror as the microscopist examines a tumor of embryonal tissue which, I think, is one of the chief reasons why the malignant potentialities of trophoblastic proliferations are so often overestimated and the lesions overdiagnosed. The pathologist must always remember that trophoblast is an embryonal tissue, that even when not neoplastic it possesses the property of invading maternal tissue and blood vessels, and therefore will combine both the architectural and cytological appearances usually associated with malignant tumors. It follows that, to some extent, one must discount the malignant appearance of trophoblastic cells and cell aggregates in many circumstances. It is at this point that terror gives way to inquisition, that is, to the process of scientific enquiry, a process of observation and ratiocination designed to analyze the properties of the tissue, to relate the present instance to past experience, and to evaluate the possible behavior of the lesion.

In so doing it must be remembered that the properties to be studied are both functional and structural, that the morphologic aspects must be placed in the framework of the clinical aspects, and that the clinical framework must be supported by correlation with the morphologic evidence of biological events. Although as individuals we may elect to focus our attention on a specific property of trophoblast or upon a specific phenomenon in a given case or series of cases, the analytical process is somewhat artificial,

and synthesis or integration is necessary before the problem can be seen in all dimensions.

One of the most frequently neglected dimensions of the scientific process is time, the relation of the present instance to past experience. This becomes particularly apparent when one deals with a lesion infrequently encountered, an instance outside of daily experience. The rarity of trophoblastic tumors and the fact that few observers have had the opportunity to accumulate wide experience with them makes it necessary to evaluate individual instances in terms of other observers' experience. There are few items in the lexicon of oncology that require more consciousness of one's debt to one's scientific predecessors than does the problem of trophoblastic tumors. On reading earlier accounts, one becomes progressively aware that all the phenomena have been described before, even those that may strike the contemporary observer as original. The rich literature on trophoblastic growths includes many instances of the bizarre and exotic as well as those that are common to the process.

The value of historical perspective is the insight it furnishes for evaluating a case at hand or, alternatively, for planning and designing an experiment intended to isolate for study one aspect of the general problem. In the following exposition I shall select certain of the more important aspects of trophoblast and determine how many are contrived corridors. I shall try also to bring into focus some of the major unanswered questions, some of the cunning passages that require illumination before one can see where they lead.

The modern history of choriocarcinoma begins in 1895 with the work of Marchand,¹ who demonstrated that this malignancy was an epithelial tumor derived exclusively from trophoblast. Although the common name for choriocarcinoma at this time was deciduoma malignum, this should not be interpreted as indicating that all authorities then believed that choriocarcinoma was derived exclusively from decidua. Even Sänger,² whose views Marchand refuted, classified tumors associated with pregnancy as "(1) sarcoma decidua-cellulare, (2) sarcoma decidua-cellulare with participation of chorionic villi, and (3) malignant interstitial hydatidiform moles and placental polyps." Gottschalk³ in 1894 dissented from Sänger's views and enunciated the doctrine that choriocarcinoma was a tumor of fetal tissue, a sarcoma of the chorion arising from the cytotrophoblast (Langhans' layer) and the stroma of the villi. Whereas Gottschalk labeled choriocarcinoma a sarcoma and considered cytotrophoblast as being of fetal mesoblastic origin, Marchand clearly stated that the tumor was derived from both syncytiotrophoblast and cytotrophoblast, both epithelial in nature. However, in his publication of 1895 Marchand adhered to the then prevalent idea that syncytiotrophoblast was of maternal origin and cytotrophoblast was of fetal origin (fetal epiblast), FIGURES 1 and 2. Subsequently, he modified this portion of his viewpoint and summarized it in 1903 in a remarkable but rarely quoted communication:⁴

"It is comprehensible that authors who first recognized tumours of this nature as something unusual, above all Sänger and Pfeiffer, should have

designated them as 'decidual sarcoma.' I may remark, historically, that to Gottschalk belongs the credit of having first rightly interpreted the masses of the so-called syncytium of the chorionic villi in a malignant tumour . . . but he erroneously regarded a part of the tumour as sarcoma arising from the stroma of the villi . . . By a specially fortunate circumstance it chanced



FIGURE 1. Reproduction of an illustration from Marchand's historical article.¹

that I was able, in a case of hydatidiform mole of the uterus, to recognize the same epithelial formations as in the two layers of the chorionic mantle, and the invasion of the muscular wall of the uterus by these cells, as in malignant chorioepithelioma. A difficulty presented itself in forming an opinion upon the origin of the tissue forms present in this growth, viz., the very diverse opinions which existed upon the two layers of the chorionic epithelium . . .

While several embryologists held both layers to be fetal, others, having in view the conditions found in animals, referred the outer layer to a maternal, the inner layer to a fetal origin. Inasmuch as at that time I had not had the opportunity to decide this question by personal study of early human ova, I had to assent to one of these views, and I felt obliged to decide upon the latter, although I had already . . . pointed out that it was often impossible to make a distinction between cells arising from the superficial and those arising from the deep layer. I regard as of the greatest importance the observation that the malignant growth actually arises from the entire epithelial covering of the villi, that at least part of the new growth . . . is of fetal ectodermal origin, a view which was later to be strenuously opposed by a number of authorities."

It may be observed in passing that in Marchand's primary communication the conclusions were based on only 2 cases that he studied personally, one a choriocarcinoma following an ectopic tubal pregnancy, the other following a normal pregnancy. Marchand reviewed 26 previously reported cases of choriocarcinoma, 13 of which developed after hydatidiform moles, 7 after term pregnancies, and 6 after abortions. Marchand also described cases of a "atypical chorionepithelioma" that now are considered examples of syncytial endometritis. At this time he had not had the opportunity of studying examples of what now would be termed chorioadenoma destruens. This lacuna was filled by the observations of Apfelstadt and Aschoff⁵ and Neumann⁶ in 1896. In reviewing cases of this period, it is sometimes difficult to classify them according to our own contemporary criteria into the tidy compartments that our present nomenclature pretends to offer. Park and Lees⁷ in their extensive review have implied that this difficulty exists. They state more explicitly the difficulties that Sanger, Gottschalk, Marchand, and Aschoff must have faced in deciding which elements were chorionic and which were decidua: "Trophoblast is quite distinctive, and so is decidua, yet if these two different and distinctive tissues are in contiguity, no sharp line can be drawn between them so that one can say that this cell or that cell is maternal or fetal."

I am sure that this difficulty has occurred to everyone who has examined placental sites with any care. The presence of Nitabuch's stria is helpful, but this boundary can be discontinuous and is of value only in intact specimens. Where there is any degree of penetration of the decidualized endometrium by proliferated trophoblast, the assignment of cells can be very treacherous indeed. Parenthetically, the presence of multinucleated cells is not helpful. While most of them are syncytiotrophoblastic, some are clearly decidua, and a few are derived from smooth muscle. Park and Lees⁷ point out that vascular endothelium, cytotrophoblast, and endometrial glandular epithelium are also potential sources for "placental site giant cells," but in practice these are uncommon sources. The contemporary morphologist faces the same difficulty as did his predecessors at the turn of the century.

Marchand's ideas were accepted with only minor controversy. In 1898, Aschoff⁸ published a fine critical *Referat* of the problem. Additional case

reports multiplied, and a further review by Münzer⁹ in 1902 served to settle the issue insofar as the German school was concerned. In 1903 Teacher¹⁰ reviewed the history of chorionic tumors, presented 3 additional cases of his own, and prepared an annotated list of 189 reported cases, all but 10 of which had been reported since 1888. Teacher's elaborate and carefully documented

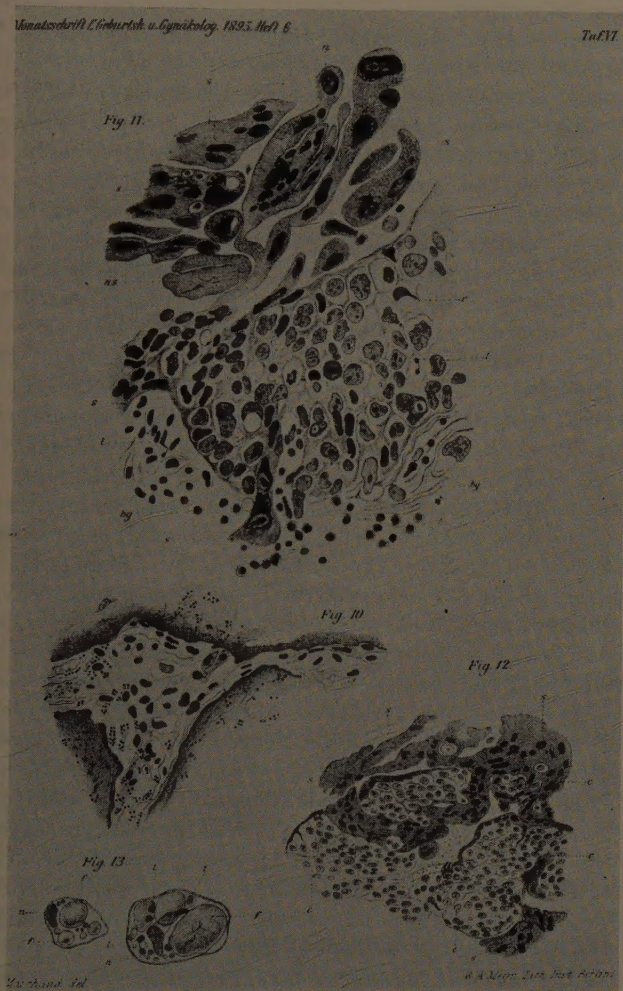


FIGURE 2. Reproduction of an illustration from Marchand's historical article.¹

study was most influential in gaining acceptance for Marchand's ideas in the British Isles as well as in the United States (which had been previously prepared for this attitude by a case report by Williams,¹¹ a study somewhat less incisive than Teacher's masterful article). In 1907, Risel¹² summarized the question and supplied an extensive bibliography. By this time there were no serious challenges to Marchand's views.

Parenthetically, Schlagenhauser¹³ reported on cases of chorionepithelioma arising in testicular teratomas. Although he subscribed to Marchand's theory that chorionic elements were fetal in origin, he went too far, interpreting some of his observations as chorionic villi and fetal membranes within the teratomas. Risel¹⁴ did not support this viewpoint in his authoritative monograph on the subject.

Once the tissue of origin was established, the next step was the taxonomy of the tumors and the other proliferations derived from it. Most of our current ideas and terminology stem from the influential publication in 1910 by Ewing.¹⁵ His opening sentences are as valid today as they were then:

"When, in 1895, Marchand demonstrated that the 'deciduoma malignum' . . . is derived from chorionic epithelium and named this tumor 'chorioepithelioma,' it was felt that not only had important progress been made in the theoretical knowledge of this disease, but that the difficulties surrounding the diagnosis and prognosis of these growths would shortly be removed. The latter hope was destined to fail . . . It became more and more apparent that tumors of chorionic epithelium exhibit a wide variation in histologic structure, that the relation between histologic structure and prognosis is extremely uncertain, and that many curious features of the natural history of these growths, especially the spontaneous recovery of apparently hopeless cases, are wholly unparalleled by other malignant neoplasms. Today it may justly be said that the study of chorioepithelioma presents more peculiar problems and its diagnosis, prognosis, and treatment encounter more special difficulties than exist with any other known tumor process."

Ewing classified all chorionic proliferations under the general term chorionoma, recognizing all the gradations of the lesion known today: hydatidiform mole, hydatidiform mole with trophoblastic proliferation, syncytial endometritis (syncytioma), chorioadenoma destruens, and choriocarcinoma (chorioepithelioma of Marchand). He employed these terms in substantially the sense that is currently in use. He recognized syncytial endometritis as equivalent to Marchand's atypical chorioepithelioma and defined it as an infiltration of the uterus with syncytial wandering cells which, although essentially benign, on occasion might simulate a tumor and merit the descriptive term syncytioma. On this subject he was in essential agreement with the previously stated views of Meyer.¹⁶ Ewing coined the term chorioadenoma destruens, and likened the lesion to a destructive placental polyp, rarely if ever producing general metastases, reproducing in rather orderly fashion all the structures of a normal villus, although he conceded that extension into the broad ligaments and vaginal metastases could be found. He emphasized the point that more importance can be attached to the histological diagnosis of these tumors than is often claimed and that certain general relations between histological structure and clinical course can be established. Meyer's¹⁷ full-length view of chorionic growths in 1930 shows that in two decades little of importance was added to the schema proposed by Ewing.

I do not propose to discuss these lesions individually; the pathological aspects are discussed in detail by R. R. Greene, W. W. Park, and N. B.

Friedman elsewhere in this monograph. As a pathologist, I can point out only the necessity for careful analysis and accurate diagnosis in individual cases. Novak and Seah^{18, 19} report that, in addition to 74 cases of choriocarcinoma received by the Albert F. Mathieu Registry, Chicago, Ill., they also received 85 cases bearing this diagnosis incorrectly, an error of more than 50 per cent. Of the 85 misdiagnosed cases, 46 were hydatidiform moles with some degree of trophoblastic proliferation, 19 were examples of syncytial endometritis, and 15 were examples of chorioadenoma destruens. Novak and Seah state: "Malignancy as it occurs in trophoblastic lesions is determined by the microscope, as it is in all fields of pathology, and not by the clinical course. For example, if a trophoblastic neoplasm is microscopically and unquestionable choriocarcinoma, but the patient gets well, the honest conclusion is that some patients with this disease survive, and the incorrect one would be the cynical statement sometimes heard that the patient could not have had a choriocarcinoma just because she got well. One might just as well deny the malignancy of innumerable cervical cancers just because they were cured by treatment."

By the same reasoning, it can be pointed out that, although chorioadenoma destruens is not a malignant neoplasm in the sense that it will produce general metastases, some patients with this lesion will die because of hemorrhage, sepsis, or perforation. If the microscopic criteria of Novak and Seah are followed closely and if adequate sampling is made of tissue for microscopic study, the percentage of correct diagnosis in cases contributed to the Mathieu Registry would rise from less than 50 per cent to well over 90 per cent.

When we turn from a consideration of choriocarcinoma to its most frequent precursor, the hydatidiform mole, we are again in the debt of Marchand,²⁰ who in 1898 disposed of Virchow's concept that a mole was a myxoma of the stroma of chorionic villi. Marchand demonstrated that the changes in villous stroma are degenerative in nature, not proliferative, and that the feature of moles that gives them their distinctive clinical and pathological behavior is attributable to trophoblastic proliferation. Obviously, such a distinctive lesion as a hydatidiform mole could not have escaped recognition by the ancients. Actually, the term *hydatid*, meaning droplike, was first used by Aetius of Amida in the Sixth Century. In his recent monograph Smalbraak²¹ has given a charming account of the delivery of the Countess of Henneberg on Good Friday in the year 1276, and it is not necessary for me to recount this singular episode. Our current ideas concerning the genesis of hydatidiform moles stem largely from the work of Hertig and Edmonds.²² Elsewhere in this monograph Edmonds discusses both old and new concepts of the genesis of moles. I shall not encroach on his domain, but merely shall summarize a well-known point of view.

Hydatidiform degeneration of the stroma of chorionic villi is a quantifiable alteration, commonplace to some degree in almost every abortus, exaggerated in molar gestations. Transitional forms between ordinary abortuses and full-blown hydatidiform moles occur. In one sense a hydatidiform mole is a malformation of the placenta, a result of the failure of continuing fetal angiogenesis. The failure of a functioning fetal vascular system leaves the

trophoblast as a semipermeable membrane, and the physicochemical gradients favor accumulation of fluid derived from maternal plasma within the villous stroma. It is the accumulation of this fluid that produces the characteristic gross and microscopic features of the malformation. McKay *et al.*^{23, 24} have shown by careful microchemical study that the fluid within molar villi represents a dialyzate of maternal plasma. Parenthetically, the traditional description of molar villi as avascular is not strictly true; on one occasion I laboriously examined 500 villi in each of 10 hydatidiform moles, a total of 5000 villi, and enumerated the number in which blood vessels could be detected. As I recall, capillaries can be seen in about 6 or 7 per cent of such villi, and an occasional vessel will contain a red blood cell. However, for all practical purposes, there is no fetal circulation and the villi are functionally avascular.

Inasmuch as the alterations in villous stroma are quantitative, the only place for qualitative change lies in the trophoblast, and the question of precisely when the qualitative change from hyperplasia to neoplasia takes place remains unanswered. If a mole is a tissue malformation in the sense of Nicholson, it has some increased facultative relation to tumor formation. Although we recognize this facultative relationship when trophoblastic proliferation is present, we are singularly ill-informed about the conditions that promote it. We have made but little progress since Teacher's statement in 1903: "I fear that, in spite of the intermediate stages between the mole and the tumor, we are not yet able to strip the mystery off the latter; we must infer that some unknown quantity has been added in those cases which develop into malignant growths."

Attempts to produce hydatidiform moles or chorionic tumors in animals bearing a hemochorial placenta have been as infrequent as they have been unsuccessful. Noyes,²⁵ who has been studying certain conditions that may influence trophoblastic proliferation, reports on them elsewhere in these pages.

For practical purposes, a number of attempts have been made to study and classify the degree of trophoblastic proliferation of evacuated molar tissue in an effort to predict which moles are more likely to eventuate into neoplastic lesions and which are likely to give no further clinical difficulty. Hertig and Sheldon²⁶ devised a six-point scale for classifying moles and were successful in segregating the innocuous from the dangerous. Unfortunately, they beclouded the issue by including their cases of syncytial endometritis and chorioadenoma destruens with the malignant tumors. However, their tabulations are so labeled that the reader may correct for this and still find a fair degree of correlation between the microscopic appearance of the trophoblast and its subsequent behavior. Some of Hertig and Sheldon's microscopic criteria will not find favor with contemporary microscopists, notably the criteria of "trophoblast invading its own stroma" and "tissue culturelike growth." However, Hertig and Sheldon are among the few writers in this day who use the term anaplasia correctly in its original sense, implying a morphologic reversion of the tissue in question to a more primitive stage in its ontogeny, and they are to be lauded for this. The grading of hydatidi-

form moles has become a somewhat controversial matter. Hunt *et al.*²⁷ have proposed a three-point scale of classification, and there is some evidence in a more recent publication by Hertig and Mansell²⁸ that these observers are currently using both systems. Needless to say, when one considers the large volume of tissue that a pathologist may receive from the evacuation of a molar pregnancy, the question of adequate sampling becomes important. The gross appearance of the vesicles and the accompanying mass of blood clot and debris does not assist in the selection of tissue for microscopic study; one must be prodigal with tissue embedded. There are occasional reports of the development of a choriocarcinoma or a chorioadenoma destruens following a mole in which little or no trophoblastic proliferation was seen; one always has the uneasy feeling that sections taken elsewhere in the tissue might have disclosed trophoblastic proliferation of a degree that would have given a clue to the subsequent course.

Novak and Seah^{18, 19} place little confidence in the grading of hydatidiform moles in an effort to predict sequelae. Their attitude reflects, I am sure, the result of a sincere effort to apply the histological criteria used in both of the systems that have been proposed. When observers of considerable experience are in diametric opposition regarding the value of a method, the truth is likely to lie somewhere midway between the two extremes. Therefore, in my own laboratory we have adopted the French Impressionist maxim, "Hydatidiform moles are not black or white; they are varying shades of pink and blue." The entire controversy smacks of being a contrived issue.

Before leaving the subject of hydatidiform moles, I shall make two analogies that I have found convenient in practice. Syncytial endometritis following a molar pregnancy is an exaggerated reaction at the placental site, depending upon the presence of retained trophoblastic cells that have infiltrated portions of the endometrium; it is analogous to the changes seen in a postabortal or postpartum endometritis in which retained placental-site giant cells are found in the curettings. Exuberant trophoblastic proliferation is greater in many hydatidiform moles than in most aborted pregnancies and certainly greater than in term pregnancies; therefore, the amount of residual trophoblast (largely but not entirely syncytiotrophoblast) is greater and the reaction to it is more intense. Chorioadenoma destruens bears the same relationship to a molar pregnancy as placenta increta does to a term pregnancy. Incretion is more probable with a molar pregnancy than with a term pregnancy, again because of trophoblastic proliferation.

Any future increase in our understanding of the behavior of trophoblastic lesions will depend upon our awareness of the fundamental biological properties of trophoblast and its products. Elsewhere in this monograph B. G. Böving discusses some of the biological properties of trophoblast and implantation; J. T. Velardo, the endocrine action of chorionic gonadotrophin; and E. Delfs, the application of quantitative studies of this hormone in problems of clinical diagnosis and management. I regret that both space and the state of our present knowledge do not permit a discussion of the steroid hormones also elaborated by the placenta.

I must comment briefly on a few of the aspects of trophoblast that have

both fascinated and baffled me. These are the interrelated problems of invasion, transport, immunity, and regression.

With respect to invasion, the affinity of trophoblast for maternal blood vessels has been noted since observers first trained their microscopes on the placental site, on trophoblastic tumors, and on the lungs of women dying during pregnancy (FIGURE 3). Invasiveness is not a property confined to neoplastic trophoblast as it is to other epithelia, unless we consider all placental tissue as a new growth; it is a property of normal trophoblast. The mechanism of invasion remains obscure. One reads remarks about the proteolytic activity of trophoblast. Is this hypothetical proteolysin an

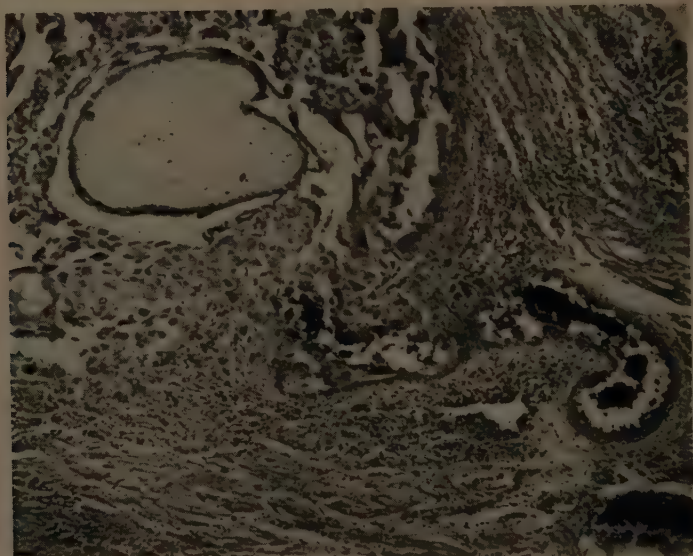


FIGURE 3. Chorioadenoma destruens showing a molar villus with proliferated trophoblast invading a vein deep in the uterine wall.

enzyme? If so, upon what specific substrate does it act? Is its action controlled or modified by any or all of the hormones elaborated by the tissue? Does it act upon maternal cells or upon the intercellular ground substance in the decidua or in blood vessel walls? It is one thing to hypothesize an enzyme; it is a different thing to isolate it. We have reached a stage of sophistication in enzymology that no longer permits us to postulate enzymatic activity with the same ease that a family doctor tells a patient that he is suffering from a virus. Unfortunately, none of the enzymes isolated so far from placental tissue appears (insofar as tested) to facilitate invasiveness. Gey and his associates^{29, 30} were able to grow trophoblast in tissue culture and demonstrate elaboration of chorionic gonadotrophin into the nutrient medium. Waltz *et al.*³¹ demonstrated gonadotrophins by culture of tissue from an hydatidiform mole even after a year of continuous growth. However, there has been no satisfactory investigation of enzymes elaborated by

trophoblast in tissue culture. Leighton's recent review³² of the contributions of tissue culture to oncology indicates that there is considerable activity in the application of this technique to the study of invasiveness by tumor cells, but to date nobody seems to have investigated the invasiveness of non-neoplastic trophoblast.

The consequence of invasion is transport. It is not difficult to visualize how trophoblastic cells that have invaded maternal blood vessels are transported to the lungs and elsewhere. This phenomenon is called physiological deportation when it involves nonneoplastic trophoblast and tumor embolization when it involves choriocarcinoma. The German term, *Verschleppung*, is particularly apposite. Schmorl^{33, 34} was able to find *verschleppte* placental cells in the lungs of 80 per cent of 158 women dying during pregnancy from a variety of causes. His observations were essentially confirmed by Veit³⁵ and Poten³⁶ who observed fragments of chorionic villi as well as isolated syncytial cells in the pulmonary vascular bed. Parenthetically, the diagnosis of deported trophoblast depends upon the demonstration of syncytiotrophoblast, not only because most of the material studied dates from the last trimester of pregnancy when cytotrophoblast has all but disappeared from the placenta, but because it would be extremely difficult to identify with certainty an isolated cytotrophoblastic cell in a pulmonary capillary.

The fate of transported trophoblast has been debated sporadically since the turn of the century. Most contemporary observers concur with Dunger's³⁷ opinion that, for the most part, *verschleppte* trophoblast in normal pregnancies survives for a limited period and then undergoes autolysis. However, trophoblastic emboli from hydatidiform moles may persist and even proliferate, elaborating enough gonadotrophin to produce a continuing positive bioassay. It is conceivable but difficult to demonstrate that such embolizations may remain latent for a period of time, eventuating in either a primary choriocarcinoma of the lung or in multiple metastases of choriocarcinoma with no evident primary site in the uterus. The circumstances under which such neoplastic transformation may occur remain undefined.

Precisely how long deported trophoblast can remain in the pulmonary vascular tree is debatable. I have studied material* from the case of a 19-year-old girl who died of subacute bacterial endocarditis with a terminal subarachnoid hemorrhage. One month prior to admission for her terminal illness she had undergone curettage for an incomplete abortion. The medium-sized and small arteries at the pulmonary apex contained viable clumps of proliferated trophoblastic cells, and there was surprisingly little cellular reaction to their presence. A few scattered trophoblastic cells were present in the regenerated endometrium as well. No trophoblast was detected in the area of cerebral hemorrhage.

A fatal outcome may ensue from embolic trophoblast that has not invaded the pulmonary parenchyma^{38, 39} if the embolization is extensive enough to produce sufficient pulmonary arterial obstruction to create either acute or chronic cor pulmonale. Harrison⁴⁰ has demonstrated this phenomenon and, through his courtesy, I have received further details of the clinical history

* Courtesy of Harold Lepow, Lincoln Hospital, New York, N. Y.

and duplicate pathological material (FIGURES 4, 5, and 6). The patient was a 47-year-old woman whose youngest child was 11 years old. Three months before admission she had aborted spontaneously and her menses had not resumed. She developed signs and symptoms of acute right-sided heart failure and pulmonary hypertension and died one month later. Autopsy disclosed that the lungs were studded with whitish plugs of embolic trophoblast. Similar emboli were found in the left ovarian vein and the paracervical venous plexus, but no residual trophoblast was found in the uterus itself. Despite careful search, at no point was invasion beyond the blood



FIGURE 4. Choriocarcinoma embolic to lung without parenchymal invasion. Large branches of the pulmonary artery are filled with embolic tumor creating pulmonary hypertension and leading to death in *cor pulmonale*. Reproduced by permission from C. V. Harrison.

vessels found in this case of choriocarcinoma. The walls of some of the pulmonary vessels harboring embolic tumor were diffusely infiltrated by inflammatory cells, somewhat reminiscent of an allergic arteritis.* Also, I was privileged to study a similar case with Hugh Grady at the Armed Forces Institute of Pathology, Washington, D. C. in which the pulmonary arterial obstruction was in the form of a large thrombus in the main pulmonary artery, its major rami not extending into the smaller vessels of the lesser

* Between the date of compilation of the monograph and the time of publication, K. D. Bagshawe and W. D. W. Brooks reported on "Subacute pulmonary hypertension due to chorionepithelioma" (*Lancet* 1: 653-658, March 28, 1959) citing this case in detail as well as one other autopsied case and one case with survival following methotrexate therapy. In these 3 cases there was no evident tumor in the endometrium, but there was extensive embolization to the lungs.

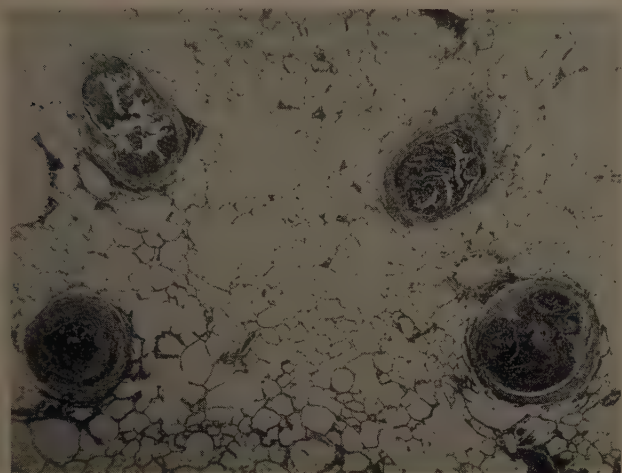


FIGURE 5. Same case as shown in FIGURE 4. Cross section of medium-sized pulmonary artery branches show the diffuseness of the embolic process. The walls of all four vessels are thickened and inflamed.

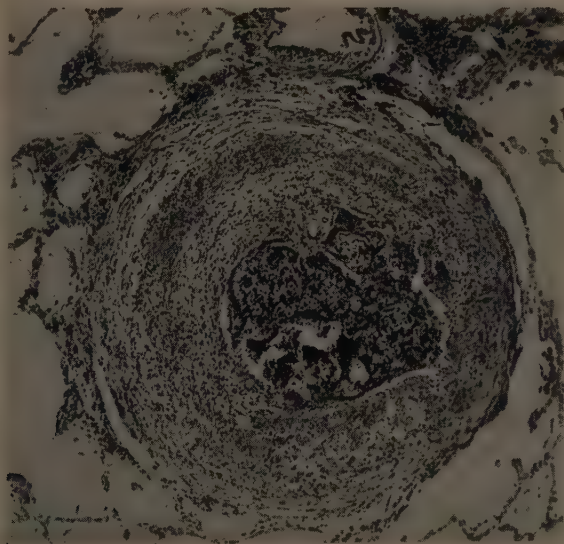


FIGURE 6. Same case as shown in FIGURES 4 and 5. Detail of a pulmonary artery branch containing embolic tumor. The entire thickness of the vessel wall is infiltrated by lymphocytes and plasma cells. In the ground substance of the media there is amorphous eosinophilic material suggesting fibrinoid.

circulation. Obstruction was incomplete and the clinical course was prolonged. On gross examination, the thrombus appeared to consist entirely of clotted blood; only on microscopic examination was it apparent that relatively small amounts of trophoblast were present near the intimal surface and that over a period of time layer upon layer of clotted blood had been deposited upon the trophoblast. Subsequent to this observation it was discovered that the patient's last-known conceptus had been more than two years prior to her death.

Recently, Park⁴¹ has investigated the results of intravenous injections of an emulsion of homologous and autologous placental tissue in mice. The injected material behaved as bland emboli with no significant inflammatory reaction, no real organization. Park⁴² has described an example of embolic decidua in the lung of a pregnant woman, and Lattes *et al.*⁴³ have reported an example of pulmonary endometriosis with decidual transformation of the stroma. The latter case occurred during the patient's second pregnancy and was interpreted as the result of embolic displacement at the time of cesarean section at the end of her first pregnancy. It is interesting to speculate on the possibility of embolic trophoblast implanting on previously embolized and viable decidua. Yet no one has successfully injected trophoblast intravenously and succeeded in making it "take." In this respect Park's results are as negative as those of Maximow (1898) and Lengemann, (1899) both of which he cites.

The question of immune responses by the maternal organism in response to the presence of trophoblast remains largely a matter for speculation. It is possible to record a long list of experimental failures starting with the pioneer work of Scholten and Veit⁴⁴ and their syncytiolysin. Elsewhere in this monograph W. A. Bardawil and B. L. Toy discuss this problem in detail. One of the chief difficulties encountered is that most of the substances prepared for experimental study have been toxic. This inevitably raises the question of the relationship of trophoblastic lesions to toxemias of pregnancy. It is readily established that there is a higher incidence of toxemia in molar than in nonmolar pregnancies. However, in the few recorded instances in which frank choriocarcinoma existed concomitant with an otherwise normal pregnancy, there has been no conspicuous evidence of toxemia. Despite the fact that immune substances have yet to be isolated from trophoblast, Schopper and Pliess^{45, 46} have drawn the analogy between the behavior of choriocarcinoma and inoculation tumors, and Schuster⁴⁷ has expanded the analogy to include tumor induced by filtrable viruses.

As a morphologist, I am tempted to think that most efforts to investigate this problem have been misdirected. In these days when there has been so much interest in tissue transplantation, I can suggest merely that the fertilized-ovum implanting is a naturally occurring instance of transplantation, a structure that is half homograft and half autograft (half autologous and maternal, half homologous and paternal). Under normal circumstances its behavior is quite predictable and quite suitable for experimental investigation. I also suggest that the placental site has the anatomic hallmarks of a field of immune tissue response. Such phenomena as fibrinoid, alteration

of the ground substance, endothelial response, focal necrosis, and a scattered inflammatory infiltrate of irregular distribution and varying composition are regularly seen. It is not beyond conjecture that the placental site contains some of the answers to problems of immunity to trophoblast. The preliminary histochemical studies by McKay *et al.*⁴⁸ offer one profitable avenue of approach.

In a wider sense, certain other questions are posed by the concept of immunity to trophoblast: Are some fertilized ova rejected for implantation, or are some implanted ova aborted as a consequence of an immune reaction? Does the onset of parturition depend in part upon some immune mechanism? If such immune mechanisms exist, are they susceptible to endocrine modification?

The questions of immunity and spontaneous regression are closely related. Frankl,⁴⁹ as well as others, has advanced the idea that the development of choriocarcinoma represents the failure of the maternal organism to develop the usual defense mechanisms against trophoblast, albeit these defense mechanisms are not defined. Presumably, spontaneous regression of an established tumor of trophoblast would indicate the development of such defenses. It is of some interest that spontaneous regression has not been reported in cases of choriocarcinoma arising in the male from testicular teratomata. This assumes special significance when viewed in terms of the success of Hertz and his collaborators^{50, 51} in treating choriocarcinoma in women with methotrexate in contrast with the failure of such therapy in teratomatous choriocarcinoma in males.

With respect to choriocarcinoma, spontaneous regression has come to mean regression of pulmonary metastases, the pathological nature of the removed primary tumor having been established at hysterectomy. It is generally accepted that the so-called vaginal metastases associated with trophoblastic tumors are usually retrograde venous embolization by trophoblast, rather than autonomous secondary deposits. Park and Lees⁷ estimate that there are about 20 acceptable cases of spontaneous regression recorded in the literature. In most of these the evidence is largely clinical; in a few cases it is supported by radiological and endocrinological evidence. Only rarely is there any microscopic confirmation of the histopathological nature of the metastases. The case recently reported by Chun and Hou⁵² is one of the best documented; although pulmonary metastases regressed, autopsy revealed that death was due to cerebral metastases. In a recently reported case Bardawil *et al.*⁵³ describe possible pulmonary metastases in a case of hydatidiform mole, evidence of regression being radiological and endocrinological. The fact that spontaneous regression (under nonspecific, symptomatic therapy) of choriocarcinoma that has metastasized can occur, albeit rarely, keeps in the foreground the question: What are the maternal defenses against trophoblastic proliferation?

Partial or complete regression of metastases has been reported following a wide range of therapeutic measures ranging from X radiation and nitrogen mustard to a variety of endocrine substances. Richter,⁵⁴ following Frankl's⁴⁹ suggestions regarding immune mechanisms, treated a patient with blood

transfusions from a pregnant woman. Unfortunately, there was no morphologic confirmation of the clinical diagnosis of choriocarcinoma (in retrospect, a diagnosis of syncytial endometritis with theca-lutein cysts is equally probable). More recently, Doniach *et al.*⁵⁵ have attempted therapy of a woman with established choriocarcinoma by immunization with her husband's leukocytes. Apparent temporary arrest of this disease was obtained but relapse necessitated the use of methotrexate. True regression was not claimed for the immunization procedures; methotrexate therapy was followed by reversion of the Hogben test to negative and radiological clearing of pulmonary densities.* The question of the mechanism of antifolic action against trophoblast is raised. The criteria for evaluating success of therapy in so unpredictable a disease as choriocarcinoma are difficult to formulate. However, most observers concur that in more than 90 per cent of the cases of correctly diagnosed choriocarcinoma death occurs within 6 months to 1 year. While any individual case, irrespective of the type of therapy, may exceed this limit, it is not unreasonable to believe that any proposed therapeutic agent when applied to a number of cases should produce a remission of greater than 1 year in a majority of cases in order to be considered an effective instrument in cancer control.

Finally, lest in our zeal as laboratory scientists we forget that trophoblastic tumors occur in human beings (insofar as I know only in human beings), this monograph includes a paper by C. L. Buxton on the management of patients. Also, we must remember that there are ecological differences among the people of this earth, even as among the animals. The new field of geographic pathology involves the study of disease in different populations; in a world in which technological advances have accelerated the mobility of peoples and the communication of ideas, ecological differences assume special significance. Therefore, this monograph also includes a paper by L. Iverson on the geographic variation in the distribution of hydatidiform moles and choriocarcinoma.

References

1. MARCHAND, F. 1895. Über die sogenannten "decidualen" Geschwülste im Anschluss an normale Geburt, Abort, Blasenmole, und extrauterin Schwangerschaft. *Monatsschr. Geburtshilfe. & Gynäkol.* **1**: 419-438; 513-560.
2. SÄNGER, M. 1893. Über Sarcoma uteri deciduo-cellulare und andere decidualen Geschwülste. *Arch. Gynäk.* **44**: 89-148.
3. GOTTSCHALK, S. 1894. Das Sarcom der Chorionzotten. *Arch. Gynäk.* **46**: 1-100.
4. MARCHAND, F. 1903. On malignant chorionepithelioma. *J. Obstet. Gynaecol. Brit. Empire.* **4**: 74-79.
5. APFELSTADT, C. & L. ASCHOFF. 1896. Über bösartige Tumoren der Chorionzotten. *Arch. Gynäk.* **50**: 511-547.
6. NEUMANN, J. 1896. Ein Fall von "malignem Deciduom." *Wien. klin. Wochschr.* **9**: 814-816.

* A communication from Doniach received just before publication informs us that following this symptom-free, Hogben-negative interval, the patient developed a cough and the Hogben test became positive once more. Following another course of methotrexate therapy, her symptoms subsided and her Hogben test again became negative. At the moment she appears to be surviving in an uneasy symbiosis between her tumor and her antifolic metabolism.

7. PARK, W. W. & J. C. LEES. 1950. Choriocarcinoma. *A. M. A. Arch. Pathol.* **49**: 73-104; 205-241.
8. ASCHOFF, L. 1898. Geschwülste. IV. Chorionepitheliome. *Ergeb. allg. Pathol.* **5**: 106-127.
9. MÜNZER, M. 1902. Chorionepithelioma malignum. Zusammenfassendes Referat. *Zbl. allg. Path. & pathol. Anat.* **13**: 197-265.
10. TEACHER, J. H. 1903. On chorionepithelioma and the occurrence of chorionepitheliomatous and hydatidiform mole-like structures in teratomata. *J. Obstet. Gynaecol. Brit. Empire.* **4**: 1-64; 145-199.
11. WILLIAMS, J. W. 1895. Deciduoma malignum. *Johns Hopkins Hosp. Repts.* **4**: 461-499.
12. RISEL, W. 1907. Chorionepitheliome, chorionepitheliomartige Wucherungen in Teratomen und chorionepitheliomähnliche Geschwülste. *Ergeb. allg. Pathol.* **11**(2): 928-1067.
13. SCHLAGENHAUFER, F. 1902. Über das Vorkommen chorionepitheliom- und traubenmolenartiger Wucherung in Teratomen. *Wiener klin. Wochschr.* **15**: 571-580; 604-606.
14. RISEL, W. 1903. Über das Chorionepithelioma und die analogen Wucherungen in Hodenteratomen. S. Hirzel. Leipzig, Germany.
15. EWING, J. 1910. Chorioma. *Surg. Gynecol. Obstet.* **10**: 366-392.
16. MEYER, R. 1906. Zur Kenntnis der benignen chorionepithelialen Zellinvasion in die Wand des Uterus und der Tuben. *Z. Geburtsh. & Gynäk.* **58**: 98-134.
17. MEYER, R. 1930. Mola hydatidiformis und Chorionepithelioma malignum uteri. In F. Henke, and O. Lubarsch, *Handbuch der speziellen pathologischen Anatomie und Histologie.* Vol. VII/1. Uterus. pp. 625-801. Springer. Berlin, Germany.
18. NOVAK, E. & C. S. SEAH. 1954. Choriocarcinoma of the uterus. Study of 74 cases from the Mathieu Memorial Chorionepithelioma Registry. *Am. J. Obstet. Gynecol.* **67**: 933-961.
19. NOVAK, E. & C. S. SEAH. 1954. Benign trophoblastic lesions in the Mathieu Chorionepithelioma Registry (hydatidiform mole and syncytial endometritis). *Am. J. Obstet. Gynecol.* **68**: 376-390.
20. MARCHAND, F. 1898. Über den Bau der Blasenmole. *Z. Geburtsh. & Gynäk.* **32**: 405-472.
21. SMALBRAAK, J. 1957. Trophoblastic Growths. A Clinical, Hormonal, and Histo-pathologic Study of Hydatidiform Mole and Choriocarcinoma. Elsevier. Amsterdam, Holland.
22. HERTIG, A. T. & H. W. EDMONDS. 1940. Genesis of hydatidiform mole. *Arch. Pathol.* **30**: 260-291.
23. MCKAY, D. G., C. C. ROBY, A. T. HERTIG & M. V. RICHARDSON. 1955. Studies on the function of early human trophoblast. I. Observations on the chemical composition of the fluid of hydatidiform moles. *Am. J. Obstet. Gynecol.* **69**: 722-734.
24. MCKAY, D. G., M. V. RICHARDSON & A. T. HERTIG. 1958. Studies on the function of early human trophoblast. III. A study of the protein structures of mole fluid, chorionic and amniotic fluids by paper electrophoresis. *Am. J. Obstet. Gynecol.* **75**: 699-707.
25. NOYES, R. W. & Z. DICKMANN. 1957. Proliferative changes in the trophoblast following ovariectomy and fetectomy in the rat. *Cancer Research.* **17**: 1054-1057.
26. HERTIG, A. T. & W. H. SHELDON. 1947. Hydatidiform mole. A pathologico-clinical correlation of 200 cases. *Am. J. Obstet. Gynecol.* **53**: 1-36.
27. HUNT, W., M. B. DOCKERTY & L. M. RANDALL. 1953. Hydatidiform mole. A clinicopathologic study involving "grading" as a measure of possible malignant change. *Obstet. & Gynecol.* **1**: 593-609.
28. HERTIG, A. T. & H. MANSELL. 1956. Tumors of the Female Sex Organs. Part 1. Hydatidiform Mole and Choriocarcinoma. Section IX—Fascicle 33. Atlas of Tumor Pathology. Armed Forces Institute of Pathology. Washington, D. C.
29. GEY, G. O., G. E. SEGAR & L. M. HELLMAN. 1938. Production of gonadotrophic substance (prolan) by placental cells in tissue culture. *Science.* **88**: 306-307.

30. JONES, G. E. S., G. O. GEY & M. K. GEY. 1943. Hormone production by placental cells maintained in continuous culture. *Bull. Johns. Hopkins Hosp.* **72**: 26-38.
31. WALTZ, H. K., W. W. TULLMER, V. J. EVANS, R. HERTZ & W. H. EARLE. 1954. Gonadotrophic hormone secretion from hydatid mole grown in tissue culture. *J. Natl. Cancer Inst.* **14**: 1173-1179.
32. LEIGHTON, J. 1957. Contributions of tissue culture to an understanding of the biology of cancer. *Cancer Research.* **17**: 929-941.
33. SCHMORL, G. 1893. *Pathologische-anatomische Untersuchungen über puerperale Eklampsie.* Vogel. Leipzig, Germany.
34. SCHMORL, G. 1904. Über das Schicksal embolisch verschleppter Plazentazellen. *Verh. deut. path. Ges.* **8**: 39-46.
35. VEIT, J. 1905. Die Verschleppung der Chorionzotten (Zottendeportation). *Bergmann. Wiesbaden, Germany.*
36. POTEN, W. 1902. Die Verschleppung der Chorionzotten. *Arch. Gynäk.* **56**: 590-617.
37. DUNGER, R. 1905. Chorionepitheliom und Blasenmole. *Beitr. path. Anat.* **37**: 279-377.
38. MARCUSE, P. M. 1953. Pulmonary syncytial giant cell embolism. Report of fetal maternal death. *Obstet. & Gynecol.* **3**: 210-213.
39. TROTTER, R. F. & H. L. TIECHE. 1957. Maternal death due to pulmonary embolism of trophoblastic cells. *Am. J. Obstet. Gynecol.* **71**: 1114-1118.
40. HARRISON, C. V. 1958. The pathology of the pulmonary vessels in pulmonary hypertension. *Brit. J. Radiol.* **31**: 217-226.
41. PARK, W. W. 1958. Experimental trophoblastic embolism of the lungs. *J. Pathol. Bacteriol.* **75**: 257-265.
42. PARK, W. W. 1954. The occurrence of decidual tissue within the lung. *J. Pathol. Bacteriol.* **67**: 563-570.
43. LATTES, R., F. SHEPARD, H. M. M. TOVELL & R. WYLIE. 1956. A clinical and pathologic study of endometriosis of the lung. *Surg. Gynecol. Obstet.* **103**: 552-558.
44. SCHOLTEN, R. & J. VEIT. 1903. Syncytiolyse und Hämolyse, ein Beitrag zur Physiologie und Pathologie der Schwangerschaft. *Z. Geburtsh. & Gynäk.* **49**: 210-232.
45. SCHOPPER, W. & G. PLESS. 1949. Über Chorionepitheliosis. Ein Beitrag zur Genese, Diagnostik, und Bewertung ektopischer chorionepithelialer Wucherungen. *Virchow's Arch. pathol. Anat. u. Physiol.* **317**: 347-384.
46. SCHOPPER, W. 1952. Das Chorionepitheliom. *Deut. med. Wochschr.* **77**: 1218-1221.
47. SCHUSTER, A. 1952. Genese und Therapie des Chorionepithelioma malignum. *Arch. Gynäkol.* **181**: 477-499.
48. MCKAY, D. G., A. T. HERTIG, E. C. ADAMS & M. V. RICHARDSON. 1948. Histochemical observations on the human placenta. *Obstet. & Gynecol.* **12**: 1-36.
49. FRANKL, O. 1937. Chorionepitheliom. Ein kritischer Bericht. *Ber. ges. Gynäk. & Geburtsh.* **33**: 385-401.
50. LI, M. C., R. HERTZ & D. B. SPENCER. 1956. Effect of methotrexate therapy upon choriocarcinoma and chorioadenoma. *Proc. Soc. Exptl. Biol. Med.* **93**: 361-366.
51. LI, M. C., R. HERTZ & D. B. BERGENSTAL. 1958. Therapy of choriocarcinoma and related trophoblastic tumors with folic acid and purine antagonists. *New England J. Med.* **259**: 66-74.
52. CHUN, D. & P. C. HOU. 1957. Spontaneous regression of pulmonary metastases in a case of chorionepithelioma. *J. Obstet. Gynaecol. Brit. Empire.* **64**: 222-226.
53. BARDAWIL, W. A., A. T. HERTIG & J. T. VELARDO. 1957. Regression of trophoblast. I. Hydatidiform mole; a case of unusual features, possibly metastasis and regression; review of literature. *Obstet. & Gynecol.* **10**: 614-625.
54. RICHTER, W. E. 1939. Transfusion von Schwangerenblut bei Chorionepitheliom. *Zentr. Gynäk.* **63**: 1567-1573.
55. DONIACH, I., J. H. CROOKSTON & T. I. COPE. 1958. Attempted treatment of a patient with choriocarcinoma by immunization with her husband's cells. *J. Obstet. Gynaecol. Brit. Empire.* **65**: 553-556.

THE BIOLOGY OF TROPHOBLAST

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Like any tissue or organ, trophoblast has certain general properties and relationships and other specific ones. My objective is to show that both should be considered in trying to describe what trophoblast is and how it works, but that general features must be well defined in terms of specific features if generalizations are to be scientifically useful.

Part I

As implied by the name suggested by Hubrecht (1888, 1889), trophoblast is primarily the nutritive tissue of the mammalian conceptus; in this function it exhibits an endodermal character. Trophoblast also has mesodermal properties, such as giving rise to the extraembryonic mesoderm in primates (Hertig, 1935; Wislocki and Streeter, 1938). Perhaps its ability to secrete gonadotrophin (Gey *et al.*, 1938; Jones *et al.*, 1943) and certainly its peripheral location may be regarded as ectodermal characteristics. Thus, trophoblast has characteristics of all three germ layers and cannot, in terms of behavior, be assigned solely to any one of them. This should not be considered surprising for, anatomically, trophoblast, in a sense, may be present before any of the three germ layers have differentiated and, in the opossum (McCrary, 1938), even before the embryo has differentiated from the rest of the conceptus. Accordingly, McCrary (1944) reevaluated the controversial interpretation of trophoblast as endodermal (Assheton, 1898) or ectodermal (Hill, 1910), and suggested that trophoblast as well as all three germ layers be considered derivatives of "protoderm."

Since trophoblast arises from tissue as generalized as that from which the embryo itself arises, due respect for the idea of ontogeny recapitulating phylogeny warns us not to assume that trophoblast is a strictly mammalian specialization. We are therefore not surprised to find eggs of lower vertebrates showing trophoblastlike behavior. When they do, as in the various placental arrangements of viviparous reptiles, amphibians, fishes, and selachians (Amoroso, 1952), it appears to be principally the nutritive function that is developed, but anchoring by fit of general contours and, in a few cases, even by interdigitation of the tissues of conceptus and mother, is achieved concomitantly in various degrees. Nevertheless, chemical exchange and anchoring are by no means as elaborately developed as in those groups of mammals whose trophoblast invades maternal tissues. Thus, from the viewpoint of comparative anatomy, the nutritive role of trophoblast is primitive, whereas invasiveness, that property of trophoblast of paramount interest in this monograph, is not a general property of trophoblast and not even a property of the trophoblast of all mammals. It is a comparatively late elaboration with many variations in anatomical detail (Hubrecht, 1889). In those species where invasion does occur, it does not necessarily begin

the instant that trophoblast appears or may be distinguished from embryo. It may lag by days, as if dependent on some form of differentiation. In the rabbit, there is even a regional difference in lag; the definitive placentation at the embryonic pole consistently begins about two days after the transitory yolk-sac placentation begins in the opposite hemisphere. Thus, from the viewpoint of developmental as well as comparative anatomy, invasiveness appears to be not so much a basic characteristic of an undifferentiated tissue as just one of many specializations of structure and function that a particular undifferentiated tissue may acquire (and later lose), if one judges from trophoblast behavior only in its normal environment.

Under abnormal conditions, trophoblast behavior suggests various and inconsistent conclusions. In tissue culture, rabbit trophoblast has a latent period of 2 to 4 days before exhibiting activity that may be compared with normal invasive behavior (Maximow, 1925). This lag, like the one in the normal environment, suggests that some form of differentiation is necessary before trophoblast can invade.

Apparently contradictory are the phylogenetic and ontogenetic implications of the important but not widely known discovery of Galtsoff and Galtsoff (1953 and elsewhere in this monograph) that 5- to 9-mm. embryos of a fish (*Opsanus tau*) become invasive when injected into the peritoneal cavity; these embryos adhere to, penetrate, and implant in the liver and body wall and become vascularized.

It could be said, of course, that a fish conceptus cannot be regarded as having any trophoblast. Nevertheless, the question of whether invasiveness is a primitive or a specialized attribute appears to have received rational phylogenetic and ontogenetic answers on both sides. A dilemma results. It is not solved by saying that trophoblastic (or malignant) invasion is concerned only with histogenetic primitiveness, for this is ultimately defined by the histological characteristics of ontogenetically primitive cells and only takes the problem on a circular sidetrack back to the dilemma. In such unresolved conflicts, significant error is less likely to lie in one or another of the answers than in the question.

What is wrong with the apparently simple question of whether invasiveness is a primitive or specialized attribute? It only appears simple; it has several hidden omissions and assumptions: (1) it permits evidence from normal and abnormal situations to be lumped, thereby assuming all invasive mechanisms in both situations to be identical without requiring the mechanisms to be identified and compared; (2) it is often tacitly coupled with the idea, questioned by Mossman (1937), that invasiveness is a solely digestive property, which ignores the possibility of noncytolytic chemical mechanisms as well as physical mechanisms; and (3) it may assume that invasiveness is solely a trophoblastic responsibility; more particularly, it may ignore possible influences from the rest of the conceptus, its metabolites, its covering membranes, the uterine secretions, epithelium, submucosa, and, by way of the blood, remote parts of the mother.

This third oversimplification is partly remedied by the traditional controversy about the extent to which the trophoblast is aggressive and the

uterus (particularly the decidual reaction) defensive, passive, cooperative, or lushly inviting. A middle position, holding neither element chiefly responsible, has been proposed and substantially supported by Fawcett *et al.* (1947).

That trophoblast invasiveness is inherent seems to be demonstrated by the fact that in certain tissue cultures, (Maximow, 1925) where maternal influences, of course, were excluded, trophoblast destroyed, or at least persisted longer than—and utilized the breakdown products of—the embryonic elements of its own conceptus. Under normal circumstances, any such inherent tendency to destroy the embryo would have to be not merely inhibited, but reversed in direction (so that maternal rather than embryonic tissues are destroyed) and even reversed in a teleological sense (so that the embryo is nourished rather than destroyed). That set of implications seems difficult enough to explain in physiological terms, even vague and hypothetical ones, but the difficulty is compounded to the point of really serious doubt by the unreasonable requirement that the implied destruction-inhibiting influence from the mother would have to act on the remote side of the trophoblast at the same time that it did not act on the side from which supplied, where it may be presumed to be more plentiful. Unless and until these unfortunate implications can be disposed of, it seems wisest to consider that the apparently destructive behavior of trophoblast evoked by the abnormal environment of tissue culture reveals potential mechanisms that are not *necessarily* the same as those that normally accomplish the anatomically orderly and rigidly scheduled processes of invasion of the endometrium.

Maternal control of implantation is implied by the fact that ovariectomy can prevent implantation. Compensation by progesterone identifies the essential factor (Allen and Corner, 1929). Still, the chain of events by which it operates remains obscure.

To the trophoblast versus uterus question, then, the only general answer that can be given with assurance with respect to normal implantation behavior is that when the conceptus is ready and the uterus is conditioned by progesterone, trophoblast somehow acquires the power to invade, and endometrium somehow acquires the power to permit that invasion and, later, one or both of these powers are lost. This is too trite and vague to be satisfying. Moreover, it becomes extremely awkward of application when due respect must be given to the detailed variations of behavior in time and place. For example (FIGURE 1), a human conceptus of about 11 days (late Horizon IV) passed through the epithelium about 5 days previously and presumably injured it, yet now, with trophoblast still in contact with it and separating it from its blood supply, the epithelium not only survives but heals the breach. The adjacent trophoblast also looks healthy and growing, a rather peaceful scene for a supposedly general battle of tissues. At the same time, the identical syncytium only a few microns away (*upper right*) is engulfing a maternal vessel while, deep in the submucosa (*lower left*), a gland is partly enveloped, and its epithelium appears injured. Since a single syncytium successively attacks and supports one maternal tissue and, while supporting it, simultaneously attacks both similar and

different maternal tissues, it seems inappropriate to attribute invasion and its regulation to any property of the trophoblast or of the maternal tissues which is general in either time or place. Thus, the question of relative responsibilities for trophoblast invasion of endometrium not only contains the several hidden oversimplifications of the trophoblast primitiveness versus specialization question, but it seeks an answer that must be inconsistent with the normal variations of trophoblast behavior in time and place.

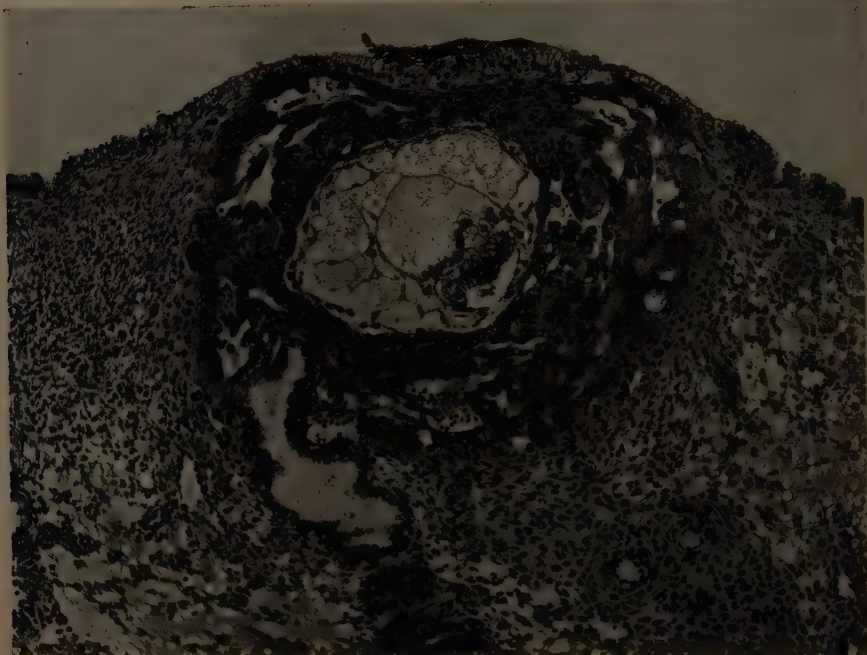


FIGURE 1. The human ovum (7699, 8-5-3), late Horizon IV about 11 days after ovulation, here appears to be covered by epithelium. Other sections show that the cover is not quite complete, but it is evident that the epithelium is regenerating rather than being destroyed by the adjacent trophoblast. Elsewhere the trophoblast is enveloping vessels (*upper and middle right*) and injuring a gland (*lower left*). $\times 100$. Reproduced from Hertig and Rock (1941) by permission from the Carnegie Institution of Washington, Baltimore, Md.

In spite of their aura of common sense, the two general questions assessing primitiveness and relative responsibility are formally two pairs of defective hypotheses. They have stimulated much conjecture and study, but the resulting discoveries have neither proved nor refuted the hypotheses, nor have the hypotheses provided good logical frameworks for relating the discoveries and progressing rationally to new ones. In other words, the hypotheses have had valuable emotional impact, but their ability to predict testable consequences has been poor. A possible reason for this shortcoming is that the hypotheses have been based on terms with inadequate definitions, and the deductions that can be drawn must remain vague until

tests of more specific hypotheses have defined the terms clearly and established their relationship. However, once such a connected account of specific mechanisms is achieved, it seems likely that any general hypotheses derived from it concerning the hierarchical nature and proportion of responsibility for invasiveness will have become relatively useless for research purposes and of only philosophical and didactic interest.

The hypothesis that there is some fundamental relation between trophoblast and cancer is another general hypothesis with emotional value, but it has so little predictive value that it cannot be directly proved or disproved. Again, there is inadequate definition of fundamental factors and their means of interaction. Consequently, we cannot judge which of known (and unknown) similarities are pertinent, and, as with the previous hypothesis, once the mechanisms and their operation become known and provide a basis for testing the general hypothesis, such a test will have become more a formality than a contribution to understanding.

The hypothesis that cancer and trophoblast are identical has also been urged (Krebs *et al.*, 1950). The identity hypothesis may appear to be merely an extreme form of the fundamental relation hypothesis, but it should be recognized that the relation hypothesis could be true even if the identity hypothesis were not, and it probably is not. As a hypothesis it has the same defects as the relation hypothesis; the components and their interplay are at present too ill-defined to permit prediction of detailed consequences. Moreover, it is less efficient to work with because it is less selective; it requires that attention be given to all features, even those that have no demonstrated relevance to invasion or malignancy. As a thesis in the logical sense of an unproved statement assumed as a premise, it yields no deductions at all so long as it stands alone. Under these circumstances the absence of disproof is in no sense equivalent to proof; it is merely a reflection of incomplete reasoning. While in this state of being neither proved nor disproved, the thesis, of course, can yield no deductions with real scientific meaning even if valid facts are used as related premises. As a thesis in the sense of a conclusion or proposition to be defended in argument, it contains serious logical faults. It asserts that two categories are identical because they have a number of similarities. It fails to apply the negative part of the test for identity, an adequate demonstration that there are no dissimilarities. There are some. As long as clinicians and pathologists can distinguish pregnancy from cancer, as long as pregnancy and cancer can coexist in one patient yet progress to different fates, and as long as the sex incidence of cancer is nearly even, while women are necessarily more often exposed to trophoblast than men, one must insist that the hypothesis of identity is too contrary to the simplest interpretations of the morphologic, the physiological, and the epidemiological facts to be accepted without proof, and that the proof thus far available has not satisfied the logical requirements for avoiding a mistaken conclusion. The relation between trophoblast and cancer remains a stimulating but unanswered question.

My criticisms may be summed up by saying that these and the previously cited general questions of primitiveness and of degree of responsibility for

invasion are not, as they may appear to be, merely modest approaches or necessary preliminaries that merely lack fulfillment; they are stimulating but unfortunately logically inadequate approaches. The inadequacy stems not from the fact that they are general but, principally, from the defect that the particulars that seem to be summarized by the generalizations are too poorly defined or related to permit detailed, secure, and worthwhile deductions.

Part II

Having criticized the existing hypotheses, it remains for me to suggest an alternative theory and to demonstrate its advantages. A suitable question, at once general and specific, is: What are the physical and chemical mechanisms of invasion and how do they work? The question may be asked of trophoblastic invasion of endometrium, and it may be asked of cancerous invasion, but definitive comparison of the two biological systems will be justified only after both questions are answered.

There seems to be some advantage in taking the trophoblast question first; trophoblast is a more convenient biological tool than cancer, because it invades on a rapid and predictable schedule and also ceases to invade predictably, an aspect one would especially like to learn to imitate for therapeutic purposes.

I shall not pretend to supply adequate answers to the question of the mechanisms of invasion; all I can offer is a collection of testable hypotheses and the results of a few tests. However, generations of rabbits have answered the questions prolifically, so it is with some humility that I select a rabbit blastocyst (FIGURE 2) to explain trophoblast invasion. This rabbit blastocyst was a sphere about 5 to 7 mm. in diameter when fresh. It had several hundred discrete points of attachment between its trophoblast and the endometrium, and these were in the hemisphere opposite the embryo, forming the temporary yolk-sac placenta. Where attachments have occurred, all conditions essential for invasion must have been met and, conversely, where there is no invasion, something essential must have been lacking. Thus, the attachments are histological pointers by which the rabbit is telling us what is necessary for invasion. Translation into anatomical and chemical terms requires only the identification of the structures and substances to which the individual trophoblastic attachments are pointing.

In an effort to avoid overlooking something, factors necessary for invasion were looked for by categories, both physical and chemical, in the conceptus and in the uterus. Moreover, mutual and mixed factors were considered, but nonlocalized factors and remote maternal factors were necessarily deferred for other methods of identification because they could not be pointed to by the invasions (Böving, in press, *a*).

Necessary physical factors of the conceptus include aggregates of trophoblastic syncytium (FIGURE 3). These so-called knobs are characteristic of the abembryonic hemisphere (FIGURE 2), but the relation to invasion is not merely regional but precise; the knobs (about 100 μ across) invade, whereas

the intervening attenuated trophoblast does not (Assheton, 1895; Schoenfeld, 1903). The slight projection and pointed shape of the knobs were interpreted by Assheton (1895) as evidence that the knobs penetrated the epithelium by being pushed through it by the hydraulic pressure of the expanding blastocyst. I note a loss of turgor of the blastocyst as a whole at the time of implantation and conceive the pointed contours of the attached knobs as less analogous to thumb tacks than to chewing gum stuck to both heel and floor. In short, the contours may be attributable to tension from

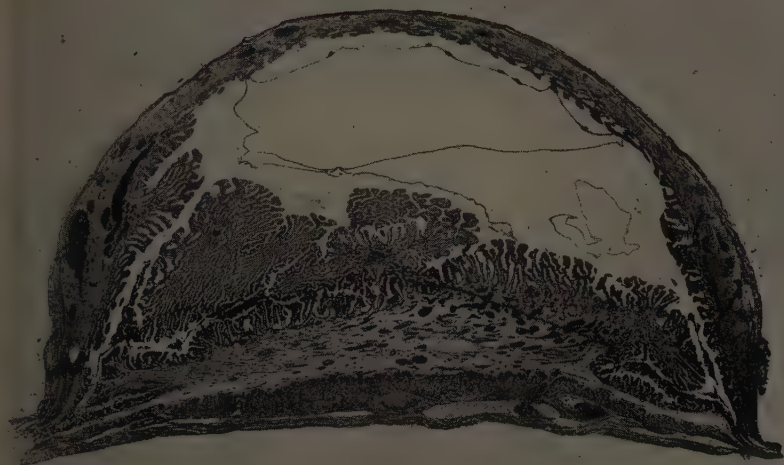


FIGURE 2. A rabbit blastocyst (L-25/25-17), 7 days after mating, is attached to the uterus at several hundred points, of which 3 appear in this section. All are in the abembryonic hemisphere and each involves a trophoblast knob. Disintegration of the non-cellular envelope, composed of mucolemma and gloiolemma, is also characteristic of the abembryonic hemisphere; the envelope is still present in the opposite hemisphere. $\times 12$.

shrinkage during histological preparation. These conflicting ideas were tested by placing nitrocellulose tetrahedrons of appropriate size in the lumen of a pregnant uterus the day before blastocyst implantation time and leaving them there for two days. The points impinged on uterine epithelium and caused compression, stretching, and tearing of tissues (FIGURES 4 and 5). Thus, the brute force of uterine contraction against an unyielding point is apparently able to force it through the epithelium. One may debate if the epithelium is actually perforated or merely attenuated and if this occurred during life or in preparation, but it is probably more important to decide if forced penetration actually occurs physiologically. More specifically, is the trophoblast knob actually an unyielding point? This seems unlikely,

because knobs are rounded before they adhere to the epithelium (FIGURE 3). Moreover, in the same uterus that housed the tetrahedrons, the gentle pressure of a small accumulation of pus caused the unattached knobs to project inward rather than outward from the blastocyst (FIGURE 3, *right*) and, in spite of this, penetration was accomplished by an immediately adjacent knob (FIGURE 3, *left*). Thus, knobs are essential, but their mode of operation is probably more subtle than merely that of a pushed wedge.

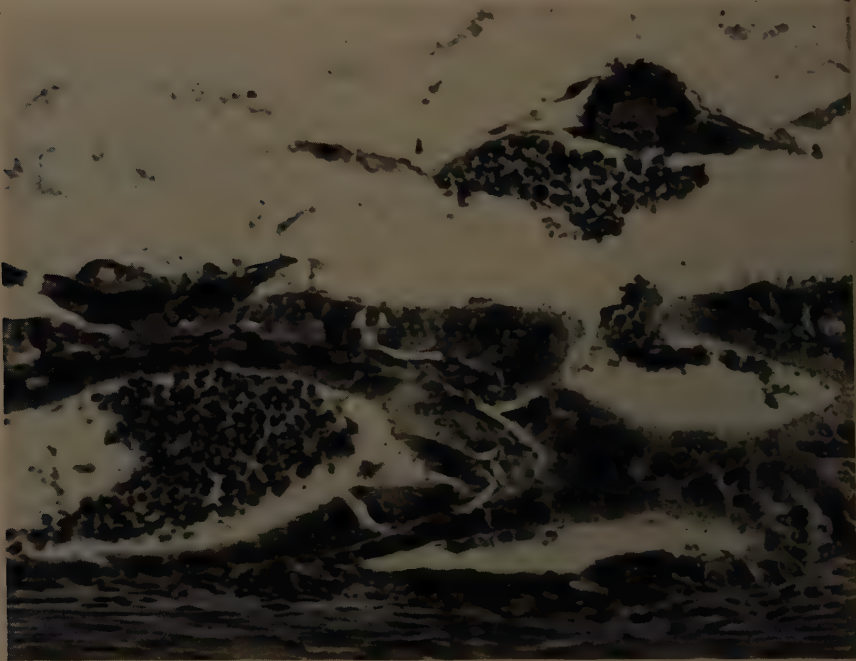


FIGURE 3. At 8 days after mating, one rabbit trophoblast knob is attached, whereas its neighbor has been pushed away from the epithelium and into the blastocyst by a small accumulation of pus. The pus is presumed to have been fluid or at least more yielding than the epithelium, since the latter is not indented (L-138-D/1-6). $\times 300$.

An essential physical factor shared by blastocyst and uterus is contact, for, of course, there can be no invasion of tissues that do not touch. In the fresh state, however, contact is quite general; contact, therefore, is not a limiting factor of practical importance (Böving, in press, *a*).

For the same reasons, subtleties of uterine contour cannot be considered a uterine physical factor. This is more specifically shown by the fact that invasions occurred at random with respect to epithelial depressions (Böving, in press, *a* and *b*). While no localized physical invasion promoter has been identified in the uterus, the existence of such a factor remains an open question.

It is probable that there is some sort of local invasion promoter in the uterus, chemical if not physical, and that, at seven days after mating, its

operating dimensions are smaller than those of the trophoblast knobs, because invasion at that time is often seen to proceed from one or more parts of a knob while other parts are not invading. Uterine chemical factors may be sought anatomically in three types of locations: sites of production, sites of storage, and sites of transfer.

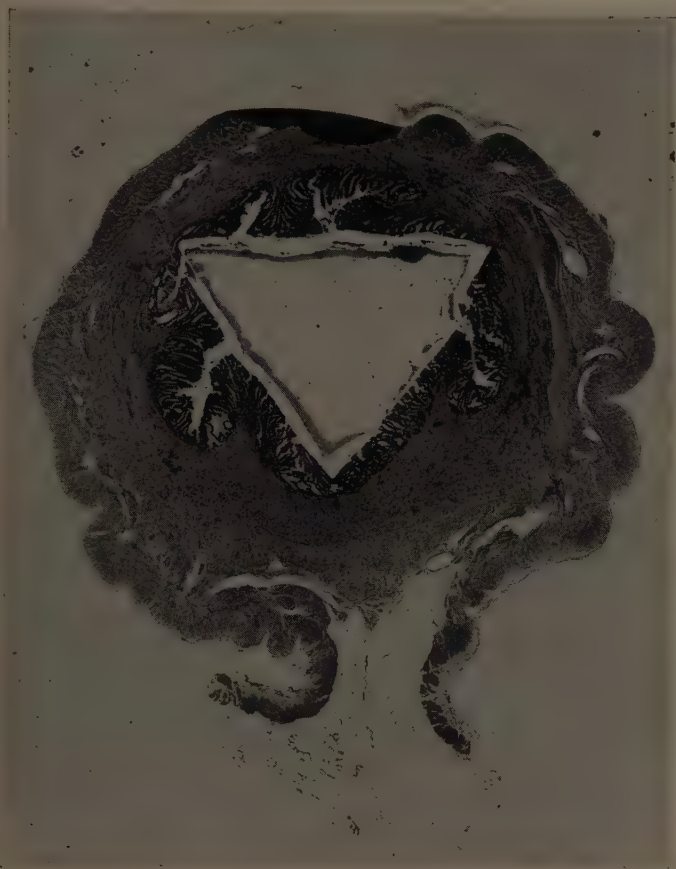


FIGURE 4. A nitrocellulose tetrahedron placed in a rabbit uterus at 6 days after mating is shown at 8 days after mating. It indented the endometrium and may have pierced the epithelium (L-138-A/1-4). $\times 12$.

I have just mentioned the fact that invasion occurs at random with respect to epithelial depressions. If these depressions are considered gland openings, one has a quantitative disproof of the idea that invasion is promoted locally by glands and their secretion. However, most of the depressions are probably not gland openings but merely folds. This alternative interpretation introduces some uncertainty, but likewise tends to disprove the idea that invasion is attributable to uterine glands and their products, although the line of reasoning is deductive rather than inductive.

It is also unlikely that invasion is promoted by anything stored in the uterine epithelium or submucosa, such as glycogen or mast cells. Actually, I have found neither glycogen nor mast cells to be anatomically associated with abembryonic rabbit trophoblast invasion, but the vast number of possible stimulators that one might think of, and the even vaster number that one might fail to think of, make it impracticable to try the specific "Can it be this?" approach to the question of stored invasion stimulators. A general although less definite answer is suggested by three considerations:

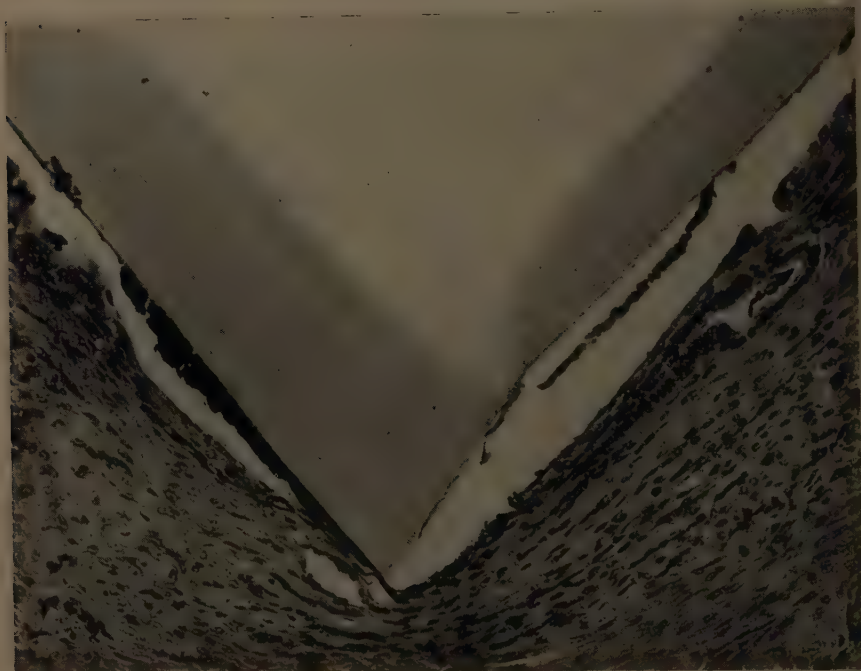


FIGURE 5. Uterine epithelium was increasingly stretched toward the angle of the tetrahedron. It is debatable whether it was attenuated or pierced at the angle and whether the effect occurred during life or during histological preparation. It is certain that there was no significant penetration of the submucosa (L-138-A/1-8). $\times 200$.

First, as distinguished from what occurs in the human, abembryonic rabbit trophoblast invasion penetrates the epithelium rapidly and then spreads at a moderate rate in the plane of the epithelium, but proceeds only slightly and slowly into the submucosa. Thus, whatever promotes invasion operates primarily in the epithelium rather than in the submucosa (but vice versa if one thinks in terms of an inhibitor).

Second, invasions that have penetrated the superficial epithelium and come in contact with the base of epithelium of deeper "glands" show no tendency to invade the base immediately. In other words, invasion is polarized; it goes rapidly from lumen to epithelial base, but not vice versa. This

is inconsistent with the idea that invasion is promoted by anything simply stored in the epithelium.

Third, invasion of trophoblast toward a stored substance requires the uncomfortable presupposition that the trophoblast can detect a static substance at a distance before it invades toward it. Thus, invasion stimulation is not readily attributable to anything stored (without spreading) in either epithelium or submucosa (Böving, *in press*, *a*).

If invasion is promoted by a substance that spreads, whether from blastocyst or uterus, then we are concerned with the site at which it is transferred from one to the other. Since implantation and even development to term may occur outside the uterus, trophoblast invasion must require nothing that is transferred only in the uterus. However, since ectopic implantation is usually unfavorable, the invasion stimulus is probably unusually plentiful or favorably disposed in the endometrium. These characteristics of distribution could apply to something carried by the maternal circulation. Accordingly, the capillaries at the base of uterine epithelium, together with the epithelium between vessel and blastocyst, were regarded as sites of transfer. This assumption was supported by finding precipitates at precisely such locations when rabbits were perfused with silver nitrate solution at seven days after mating (Böving, 1954). Trophoblast invasions were found to be centered on such sites of chemical transfer and have the same mean width (Böving, *in press*, *a*). Therefore the sites of transfer, as defined above, must be necessary for abembryonic trophoblast invasion in the rabbit.

Identification of the chemical transferred may be aided by knowing its source or direction of transfer. Since silver precipitates occurred principally in the embryonic hemisphere of blastocysts and in adjacent uterine epithelium, but did not occur in the epithelium of deep glands nor in the submucosa (FIGURE 6), the blastocyst must be the source of the substance transferred, its transfer must be principally through the abembryonic hemisphere, and it must be removed by the most accessible maternal vessels.

Thus, summarizing the anatomical analysis, abembryonic rabbit trophoblast invasion of antimesometrial uterine epithelium is promoted locally by contact between a trophoblast knob and uterine epithelium with a vessel beneath. At that site, invasion is promoted by a chemical that is produced in the blastocyst and transferred to the maternal circulation; it is not locally promoted by products secreted or stored in the endometrium.

A chemical approach is necessary to identify the substance transferred, and a physical interpretation is necessary to explain how it promotes invasion.

A starting point is provided by the abembryonic location of the invasion-promoting substance and by the fact that it precipitates silver. In addition, the absence of silver precipitates from a dead blastocyst, in contrast to heavy precipitates in an adjacent living one (FIGURE 6), suggested that a metabolic product rather than a stored one was involved. A blastocyst dried on its side acquired many crystals in its abembryonic hemisphere. Similar crystals formed from fluid that leaked from the interior. Such crystals were identified as, principally, sodium and potassium chlorides with

a small amount of calcium carbonate. Of these, carbonate seemed, more probably than chloride, to be a metabolic product. This choice was checked by placing a fresh preimplantation blastocyst in saturated lead chloride solution with some phenol red. An alkaline reaction was revealed in a region whose center was about 40° from the abembryonic pole (FIGURE 7). Lead was precipitated, presumably as the carbonate, certainly not as a chloride.

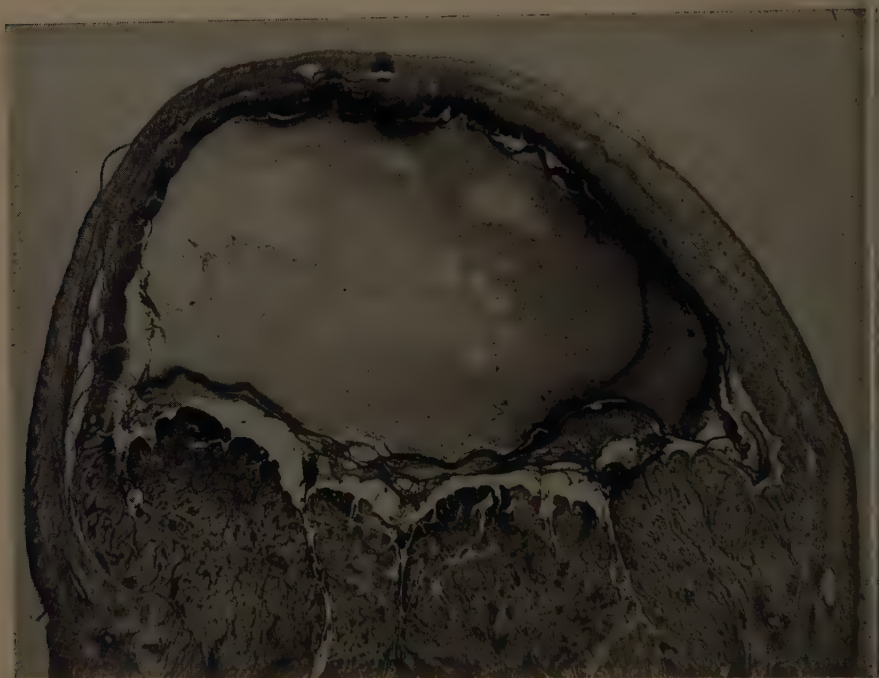


FIGURE 6. A transversely sectioned rabbit uterus 8 days after mating has black precipitates antimesometrially and in the adjacent abembryonic part of the blastocyst following perfusion with silver nitrate solution. Other regions are yellow or brown. The restricted location of the black precipitates suggests that they are caused by something coming out of the abembryonic hemisphere of the blastocyst. The absence of precipitates from the dead blastocyst (*extreme right*) suggests that the silver-precipitating agent is a metabolic product rather than a stored substance (206-C/2-2). $\times 15$.

in the same general region as the alkaline reaction (this location was subsequently confirmed more precisely when the blastocyst was placed in ammonium sulfide solution). For these reasons, the invasion-promoting substance from the blastocyst was identified as some form of carbonate. It is not dissolved carbon dioxide, since no bubbles formed when a blastocyst was subjected to low pressure (Böving, in press, *b*). A titration curve (Böving, 1954) and the consideration that the pH is about 7.3 in the uterus (Böving, 1952) and probably near that in the blastocyst suggest that, under normal circumstances, the substance is probably a bicarbonate, but that it exists at that region of its double dissociation curve where the loss of very little

acid results in a fairly rapid rise in pH , as the bicarbonate is converted to a carbonate (Böving, 1954, and in press, *b*).

The identification was checked and the mode of action was explored by tying one uterine horn of a pregnant rabbit into four compartments and, at 7 days after mating, injecting the first with sodium bicarbonate (0.15 M), the second with CO_2 -charged water, the third with sodium chloride (0.8 per

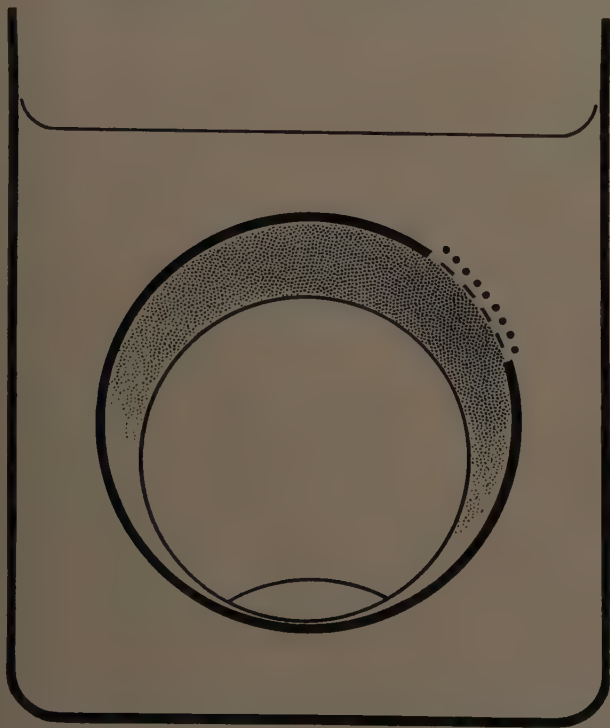


FIGURE 7. This diagram represents information originally recorded by several sequences of motion pictures. A blastocyst about $6\frac{3}{4}$ days old, 5 mm. in diameter, and nearly ready to attach to the uterus has been transferred to a vessel of test solution. The cellular elements of the blastocyst (*inner circle*) have become slightly separated from the surrounding noncellular envelope. This envelope, consisting of mucolemma and variable amounts of the gloiolemma, is especially permeable (or perforated?) in one or more regions of the hemisphere opposite the embryonic disk. Indicator dyes enter such regions and show that the pH rises to a maximum there, both inside and outside the envelope. Lead and silver are precipitated with a similar distribution.

cent) and the last with sodium hydroxide (0.05 N), 0.6 ml. each. Promptly thereafter the animal was perfused with flushing solutions (NaCl, 0.8 per cent and $NaNO_2$ 0.1 per cent; then sucrose 0.25 M) and then with silver nitrate solution (0.5 per cent). Following treatment with a photographic developer, 0.1 per cent Amidol, epivascular precipitates of silver resembling those adjacent to blastocysts were found in the "carbonate"-containing segments, that is to say, the one containing sodium bicarbonate and the one

with carbon dioxide. The precipitates from bicarbonate were as dense as those from blastocysts; the carbon dioxide precipitates were lighter. Epithelial dissociation was found in the alkaline segments (containing sodium bicarbonate or sodium hydroxide); no precipitate occurred in the segment containing sodium chloride. (It should be noted that this is a single experiment and that it requires replication with an arrangement that separates the two carbonates as well as the two alkalies, thus eliminating leakage past the ligatures as a conceivable source of error.) It was tentatively concluded that the silver precipitates were silver carbonate, that the precipitates formed by normal blastocysts reflect the carbonate aspect, and that the accompanying high pH is the aspect responsible for dissociation of the uterine epithelium (Böving, in press, *b*). If it is true that the cells of the epithelium overlying vessels become separated by the alkalinity elicited there, it is singularly appropriate that the invading trophoblast should be protected against dissociation by having a syncytial structure. Thus, invasion need not be attributed to phagocytosis, for which there is no histological evidence at this stage of implantation. It may be attributed better, in part, to the recognized physicochemical mechanism of cellular disaggregation by high pH (Holtfreter, 1944; Steinberg, 1958); this is consistent with histological features (Böving, 1958), as well as with the chemical evidence just presented. It is conceivable, but unproved, that the later disintegration of blood vessels enveloped by trophoblast may depend on the same mechanism.

The above must be only a partial explanation. Epithelial disaggregation can be expected only to clear a path for invasion; there must also be a push to send the trophoblast along that path. The knobs of the rabbit trophoblast may have a special responsibility for this. My own studies have not gone beyond histological impression and do not yet deserve further comment. For the time being, it must suffice to mention that high pH , in addition to disaggregating cells, also stimulates pseudopod formation (Holtfreter, 1944); and that pseudopodia have been observed at the implanting pole of guinea pig blastocysts kept in tissue culture (Blandau and Rumery, 1957).

If epithelial disaggregation and perhaps other effects of bicarbonate-induced high pH are essential mechanisms of rabbit trophoblast invasion, one may ask next how they are produced and regulated. In particular, why does all the bicarbonate pile up in the blastocyst, and what triggers its release and the accompanying pH rise?

About 4 days before implantation, when the rabbit conceptus becomes a blastocyst, it acquires a capacity for anaerobic glycolysis. By about 2 days before implantation, it has experienced a two-hundredfold increase in metabolic rate; this doubles the day before implantation and falls thereafter (Fridhandler *et al.*, 1956 *a*, *b*, and 1957). It has not been proved directly, but there are reasonable anatomical and chemical implications indicating that the rabbit blastocyst's chemical exchange, previously adequate for respiration, becomes temporarily overburdened under normal conditions *in utero*. During the period of respiratory inadequacy, the blastocyst may be presumed to resort to the recently acquired faculty of anaerobic glycolysis. The glycolysis would produce lactic acid and the lactic acid would depress

the pH of the blastocyst even more than would the carbonic acid from the oxidative metabolism. The lactic acid would also drive carbon dioxide from the bicarbonate that Lutwak-Mann and Laser (1954) showed to be present. No gas is evident and, while there is a decrease in the concentration of bicarbonate (Lutwak-Mann and Laser, 1954), the amount of bicarbonate per blastocyst increases the day before implantation (Böving, in press, *b*). Moreover, the macroscopically prevailing pH of the blastocyst *in utero* is held at about 7.3 to 7.8 rather than depressed. These phenomena could be explained if an alkali reserve were operating as calcium carbonate does in a number of similar biological situations, as in the clam, whose shell is eroded while it maintains pH at tolerable levels against tremendous accumulations of lactic acid and carbon dioxide, with a consequent accumulation of lactate and bicarbonate (Dugal, 1939; von Brand, 1946).

What then reverses the preceding mechanism and releases the accumulated bicarbonate (and the consequent pH rise) as implantation becomes imminent? To a slight extent, the release is almost certainly aided mechanically by the perforation of the noncellular membranes that surround the blastocyst, but it is suggested that, in the rabbit, the critical regulator is enzymatic. An arresting aspect of the four-segment experiment, mentioned previously, is that precipitates resembling those from blastocysts and bicarbonate solutions were obtained when only carbon dioxide-charged water was instilled. Since there was no precipitate in the lumen, and since carbon dioxide does not cause precipitation when bubbled through silver nitrate solution, it suggests that there was rapid and massive conversion of carbon dioxide to carbonate ion within the uterine epithelium. This is considered a manifestation of carbonic anhydrase activity, and is consistent with the biochemical demonstration by Lutwak-Mann (1954) that this enzyme is greatly augmented in rabbit endometrium shortly before and during the time of implantation. A histochemical test for the enzyme, the cobalt chloride variation of Kurata's method mentioned by Gomori (1952), gave results that were probably consistent although not conclusive (Böving, in press, *b*). The increase of carbonic anhydrase is due to progesterone (Lutwak-Mann, 1954; Adams and Lutwak-Mann, 1955).

The foregoing observations and reasoning may be condensed into an hypothesis of abembryonic rabbit trophoblast invasion. One or two days before implantation, blastocyst metabolism increases so greatly that the previously adequate respiratory mechanisms become insufficient. Glycolysis relieves some of the energy need, but metabolic wastes are produced faster than ever, and some accumulate as bicarbonate, thanks to a buffer system. As the time of implantation nears, progesterone augments endometrial carbonic anhydrase and thereby indirectly accelerates the removal of the accumulated bicarbonate by speeding carbon dioxide "blow off" from carbonic acid within the epithelial cell (FIGURE 8). As the carbon dioxide is carried off by the maternal circulation at the base of the cell, alkaline products are left behind. The consequent local pH rise causes disaggregation of the epithelial cells in which it occurs, while the adjacent trophoblast, being syncytial, remains intact and thus replaces the epithelium.

The hypothesis may be both checked and extended by some of its implications.

Since the transfer of bicarbonate is restricted to the cells with a vessel at their base at seven days after mating, the precise aim of invasions for vessels is explained when it is added that alkalinity makes the blastocyst sticky (Böving, 1954, 1958).

A second implication of the hypothesis is that the spread of invasions in the plane of the epithelium and the generalized adhesion of abembryonic blastocyst to endometrium that occur about eight days after mating are inconsistent with the sharp localization of chemical transfer across the epithelium which occurs at seven days after mating. The apparent contradiction proved to be a confirmation of the hypothesis, when silver nitrate

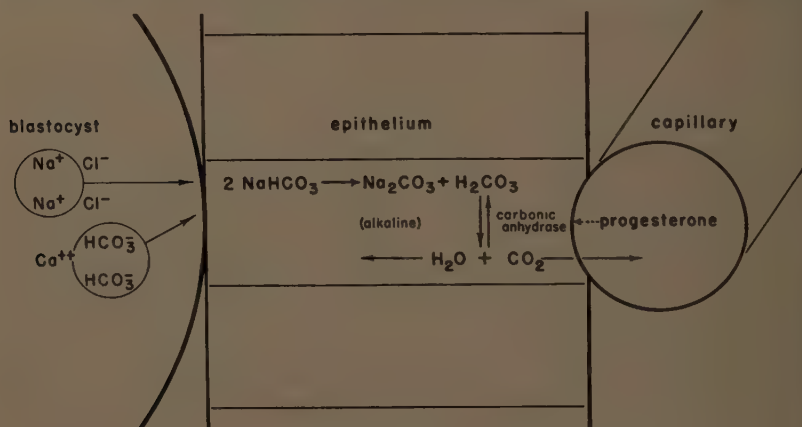


FIGURE 8. The transfer and "blow-off" phases of the proposed hypothesis are here illustrated with sodium accompanying the bicarbonate, but it has not actually been determined what metallic ion or ions are involved.

perfusions done at eight days after mating showed that bicarbonate transfer was then no longer channeled, but was diffuse throughout the epithelium. Since the intercellular membranes of the uterine epithelium disappear at this time, one may attribute to them the earlier restriction of chemical transfer and the consequent precision of trophoblastic "aim" for blood vessels (Böving, 1955).

A third implication of the hypothesis is that, as the carbon dioxide blows off with enzymatic assistance and is carried away by the maternal circulation, the alkalinity released at the site of transfer should exert a self-potentiating or snowballing effect on the reaction for, as pH rises from 6.5 to 10, the activity of carbonic anhydrase increases fivefold (Colowick and Kaplan, 1955). The pH rise in implantation is from about 7.5 to 9.5. Thus, once begun, epithelial penetration should go increasingly rapidly. This is confirmed histologically. Of 94 sections of invading knobs, 40 were no more than adherent to the epithelial surface, 37 had completely penetrated, and only 17 appeared to be just part way through the epithelium. When

the material was reconstructed and mapped, it became evident that most of the 17 partial penetrations were actually peripheral parts of knobs that had penetrated completely in some other plane of section. Only 2 of the 94 data represented a knob that was truly in an intermediate stage of penetration (Böving, in press, *a*). This shows that penetration, once begun, is rapid and not probing.

A fourth implication of the hypothesis is that, since progesterone augments carbonic anhydrase, if silver precipitates depend on and reveal that enzyme's help in transferring bicarbonate by speeding the conversion of its products, then silver perfusion should yield precipitates like those of pregnancy when performed on progesterone-treated spayed rabbits whose uteri have been charged with a bicarbonate solution of appropriate concentration. It does so (Böving, in press, *b*). A converse implication is given by the depression of endometrial carbonic anhydrase by estrogen (Adams and Lutwak-Mann, 1957). Silver perfusion of estrogen-treated spayed rabbits whose uteri have been charged with bicarbonate solution should not give precipitates such as those of pregnant or progesterone-treated rabbits. It does not (Böving, in press, *b*). Moreover, estrogen inhibits implantation and does so in very small doses, but this may involve several mechanisms other than, or in addition to, those discussed here (Greenwald, 1957).

A sixth implication is that implantation should be inhibited by the specific carbonic anhydrase inhibitors, Diamox (sodium acetazolamide) and sulfanilamide. This has not been realized, but neither was the silver precipitation reaction completely inhibited by Diamox. The specific inhibitors, while effective *in vitro*, are insufficiently effective *in vivo* within dose limits tolerable to the living animal or even at the high dose (about 2 gm. of Diamox) supplied by perfusion in an acute experiment (Böving, in press, *b*). Endometrial carbonic anhydrase was depressed no more than 50 per cent one hour after rabbits received 80 mg./kg. of Diamox intravenously at 8 days after the induction of pseudopregnancy by gonadotrophin (Lutwak-Mann, 1955).

A seventh implication follows from the preceding. One must doubt, yet recognize, the possibility that trophoblastic tumor inhibition by folic acid analogues, such as methotrexate, might be aided by carbonic anhydrase inhibition by that residue of the compounds that resembles (Sumner and Somers, 1953) sulfanilamide.

An eighth implication is that the establishment of contact with maternal circulation sufficient to relieve the deficiency of gas exchange may be regarded as a means of "turning off" the invasion mechanism. The abembryonic rabbit trophoblast knobs do, in fact, appear to be stopped where they have reached, or surrounded, vessels. This is true also in other species (Enders *et al.*, 1958). The failure of human benign trophoblastic emboli to penetrate the walls of vessels in which they lodge (Park, elsewhere in this monograph) may also illustrate this implication.

Since some aspects of the proposed mechanisms have been studied in only single experiments, my suggestions are tentative rather than firm conclusions; nevertheless, the hypothesis of abembryonic rabbit trophoblast invasion that has been outlined is specific enough to have detailed and testable impli-

cations, and the few but varied tests that have been made have supported it. In addition, this hypothesis fits not only abembryonic rabbit trophoblast invasion but also much of what little we know or may infer about invasion by cancer and by the toadfish embryos studied by the Galtsoff and Galtsoff (1953). Therefore, as far as it goes, it is more workable and more unifying than the hypotheses criticized earlier.

The hypothesis, however, is no general hypothesis of invasion. A general hypothesis would require that all of the same mechanisms be demonstrated (1) in invading toadfish embryos, (2) in all the varieties of cancer, and (3) in the trophoblastic invasion of all species where invasion occurs. Such a degree of similarity is not likely to be found. The anatomy of placentation varies greatly among the different groups of mammals (Mossman, 1937); the occurrence of endometrial carbonic anhydrase is also variable (Lutwak-Mann, 1954; Lutwak-Mann and Averill, 1954), and most invasive tumors, unlike trophoblast, are cellular rather than syncytial. Thus, the question of a relationship between trophoblast and cancer has not been answered with even a reasonably substantial hypothesis, but only with questions more specific than previous ones.

Part III

A general hypothesis of invasion is at present attainable only by speculation two "generations" removed from experimental data. The likelihood of error in such speculation is great, but the possibility of gaining ideas leading to correct understanding is also present. To avoid throwing the baby out with the bath water through fear of making a mistake, the customary propriety of refusing to speculate (or admit it) is set aside for the remainder of this paper.

Either the various instances of invasion of one tissue by another have one or more mechanisms in common, or they have not. An unsuccessful search for common mechanisms cannot be considered to prove the negative, because one cannot expect to think of everything. A successful search, however, disproves the negative, and the common mechanism revealed by it can serve as part of a general hypothesis of invasion to the extent that its operation is understood.

Dissociation of cells may be such a common mechanism; it is exhibited by cancer cells (Coman, 1944) and by the epithelium invaded by trophoblast (Part II). This mechanical resemblance may be suspected of depending on comparable, though not necessarily identical, chemical mechanisms.

Malignant tissues characteristically metabolize rapidly, yet respire at rates comparable only with normal tissues (Weinhouse *et al.*, 1956) or less (Warburg, 1956; Warburg in Weinhouse *et al.*, 1956; Burk, 1937, 1939; Burk and Schade in Weinhouse *et al.*, 1956). Thus, respiration is never sufficient to supply all of the energy that can be used. Glycolysis provides a supplement of energy; this occurs even when oxygen is present and utilized at rates that would prevent glycolysis in normal tissues. High metabolic rate, glycolytic ability, and respiration insufficient for the needs are probably also characteristic of the rabbit blastocyst under normal circumstances *in utero* during that

brief period before and during implantation when the respiratory mechanisms are insufficient for the needs (*Part II*). If that metabolic situation and compensatory pH regulation by an alkali reserve are responsible for charging the cell-dissociating, alkali-release mechanism of rabbit trophoblast invasion, then one may consider it possible that similar chemical mechanisms, which are characteristic of cancers and not of normal tissues, are also responsible for the invasion by cancers. Since cell dissociation by high pH and low calcium have a different basis (Steinberg, 1958), the suggested hypothesis may be contradicted by or may supplement Coman's (1944) attribution of invasiveness of cancers to their low calcium content. In either case, a preliminary examination seems worthwhile.

If the hypothesis is correct, then trophoblastic and cancerous invasions resemble each other in depending on high metabolism and glycolysis, but they differ in that the respiratory insufficiency of cancers is permanent, intrinsic, and not subject to external control, whereas the respiratory inadequacy of the trophoblast is temporary and extrinsic, being normally relieved by the trophoblast's physiological objective, the maternal circulation.

Warburg (1956) indicated that a temporary inadequacy of respiration might cause permanent damage to the respiratory mechanism by depriving it of the energy necessary to maintain itself. In respect to this, it is curiously appropriate that the rabbit conceptus develops considerable glycolytic ability, an alternate energy source, on becoming a blastocyst (Fridhandler *et al.*, 1957), several days *before* the inferred period of respiratory inadequacy.

The glycolytic ability of trophoblast may be a clue to the unusually rapid course of choriocarcinoma. The fact that this tumor is not arrested by maternal circulation suggests that it is not essentially different from other cancers insofar as it has respiration permanently insufficient for its needs, but it may have a head start in glycolysis. Cancers are believed to originate from other tissues by a partial injury to respiration followed by a latent period to select and build up a population of cells with accentuated glycolytic ability (Warburg, 1956). In contrast to this, choriocarcinoma arises from chorion, a tissue that already has great glycolytic ability as well as a very rapid metabolism (Burk, 1939; Warburg in Weinhouse *et al.*, 1956). Thus, its more rapid development might depend on only a partial but permanent intrinsic respiratory injury, a one- rather than a two-step genesis.

If the speculative picture thus far sketched may be considered to illustrate both ends of the malignancy spectrum of trophoblast and its tumors, then it may be subjected to a preliminary test for internal consistency by attempting to fit into it the hydatidiform moles and the curious fact that these exhibit various degrees of invasiveness, yet lack the autonomy of choriocarcinoma and other cancers. If their trophoblast may be assumed to glycolyze, as other trophoblasts do, then one is encouraged to look for a respiratory mechanism as the regulator of their invasion. A poor vascular supply is, indeed, a well-known characteristic of moles (Hertig and Edmonds, 1940), but there is more epithelial proliferation near whatever little blood supply is present (Alter, 1946). Thus, there is good morphologic reason to suppose that moles have a respiratory deficiency and that it is probably not

intrinsic to the cells, as in cancers, but extrinsic, as in the rabbit trophoblast. This is consistent with the lack of autonomy. One would expect that invasion by moles could be "turned off" by sufficient access to maternal circulation as well as by decreased metabolism or death of the trophoblast. The more subtle variations in degree of invasiveness found even in a single case of hydatidiform mole (Smalbraak, 1957 and elsewhere in this monograph) conceivably also could be explained by local variations in the ratio between metabolic rate and respiration, and it seems not unfair to suggest that these local variations of invasiveness found in normal implantation (FIGURE 1) may have a similar basis.

Some of the difficulty in understanding the mechanical behavior of trophoblast, its tumors, and other tumors as well, probably may be traced back to the dependence of invasion on a balance of several variables: total metabolism, respiration, and excretion. One would expect the balance to be more difficult to maintain than in normal tissues, because the variables are not mutually dependent to the same degree. The greater independence may be attributed to enhanced glycolysis, which lets the total metabolism escape from the control which, in normal tissues, the vascular mechanisms exert on respiration and, in turn, on total metabolism, thus keeping the output of metabolic waste products within the capacity of the normal removal mechanisms. Enhanced glycolysis of various degrees may also be suspected of underlying the compensation of cancers for regressions, whether spontaneously or therapeutically induced by way of damage to intact or already damaged respiration. Thus, while excessive metabolism and accumulation of its waste products may be essential for invasion, these, in turn, depend on enhanced glycolysis; for this reason it is suggested that glycolysis be considered the ultimate invasion-promoting factor. The balance concept encompasses the carcinogenic potential of both respiratory depression (Warburg, 1956) and of cytologically specific stimulators such as certain hormones (Woglom, 1947). The attribution of invasion to cell dissociation by the discharge of accumulated metabolic waste products suggests that control of invasion may be sought through control of the discharge (as, for example, in preventing rabbit implantation by inhibiting the progesterone-stimulated carbonic anhydrase with estrogen). However, such control is feasible only in special situations, and ultimate general control of invasion, both trophoblastic and malignant, would appear to lie in inhibiting glycolysis without inhibiting respiration. Exactly such specific inhibition and consequent specific destruction of cancer cells has recently been accomplished by Papaconstantinou and Colowick (in press) and may be regarded as the most logical as well as the most exciting recent development in the field of invasive growth control, although the approach is not without danger (Woglom, 1947).

Summary

In *Part I* several hypotheses of invasion are criticized.

In *Part II* a hypothesis explaining the invasion of abembryonic rabbit trophoblast by chemical and physical mechanisms is derived and tested.

In *Part III* similarities and differences between trophoblastic and malignant

nant invasion are speculated upon, and a general hypothesis of invasion is proposed.

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References

- ADAMS, C. E. & C. LUTWAK-MANN. 1955. Endometrial carbonic anhydrase as hormone indicator for progesterone and related steroids. *J. Endocrinol.* **13**: xix-xx.
- ALLEN, W. M. & G. W. CORNER. 1929. Physiology of the corpus luteum. III. Normal growth and implantation of embryos after very early ablation of the ovaries, under the influence of extracts of the corpus luteum. *Am. J. Physiol.* **88**: 340-346.
- ALTER, N. M. 1946. Histogenesis of hydatidiform mole. *Am. J. Pathol.* **22**: 638-639.
- AMOROSO, E. C. 1952. Placentation. In *Marshall's Physiology of Reproduction*. II. 3rd ed. A. S. Parkes, Ed. Longmans, Green. London, England; New York, N. Y.; Toronto, Canada.
- ASSHETON, R. 1895. On the causes which lead to the attachment of the mammalian embryo to the walls of the uterus. *Quart. J. Microscop. Sci.* **37**: 173-190.
- ASSHETON, R. 1898 (in 1899 vol.). The segmentation of the ovum of the sheep, with observations on the hypothesis of a hypoblastic origin for the trophoblast. *Quart. J. Microscop. Sci.* **41**: 205-262.
- BLANDAU, R. J. & R. RUMERY. 1957. Observations on the activity of the cells of the abembryonal pole in the living 6-day old guinea pig blastocyst. *Anat. Record.* **127**: 491.
- BÖVING, B. G. 1952. Internal observation of rabbit uterus. *Science.* **116**: 211-214.
- BÖVING, B. G. 1954. Blastocyst-uterine relationships. *Cold Spring Harbor Symposia Quant. Biol.* **19**: 9-28.
- BÖVING, B. G. 1955. Uterine epithelial cells guide trophoblast hemotropism. *Anat. Record.* **121**: 426.
- BÖVING, B. G. 1958. Implantation. *Ann. N. Y. Acad. Sci.* **75**: 700-725.
- BÖVING, B. G. Anatomical analysis of rabbit trophoblast invasion. *Contrib. to Embryol. Carnegie Inst. Wash.* **37**. In press, *a*.
- BÖVING, B. G. Endocrine influences on implantation. In *Endocrinology of Reproduction*. Academic Press. New York, N. Y. In press, *b*.
- BURK, D. 1937. On the biochemical significance of the Pasteur reaction and Meyerhof cycle in intermediate carbohydrate metabolism. *Am. Assoc. Advancement Sci. Symposium. Science.* **85**(Suppl.): 121-161.
- BURK, D. 1939. A colloquial consideration of the Pasteur and neo-Pasteur effects. *Cold Spring Harbor Symposia Quant. Biol.* **7**: 420-459.
- COLOWICK, S. P. & N. O. KAPLAN. 1955. *Methods in Enzymology*. Academic Press. New York, N. Y.
- COMAN, D. R. 1944. Decreased mutual adhesiveness, property of cells from squamous cell carcinomas. *Cancer Research.* **4**: 626-629.
- DUGAL, L. P. 1939. The use of calcareous shell to buffer the product of anaerobic glycolysis in *Venus mercenaria*. *J. Cellular Comp. Physiol.* **13**: 235-251.
- ENDERS, A. C., G. D. BUCHANAN & R. V. TALMADGE. 1958. Histological and histochemical observations on the armadillo uterus during the delayed and post-implantation periods. *Anat. Record.* **130**: 639-658.

- FAWCETT, D. W., G. B. WISLOCKI & C. M. WALDO. 1947. The development of mouse ova in the anterior chamber of the eye and in the abdominal cavity. *Am. J. Anat.* **81**: 413-443.
- FRIDHANDLER, L., E. S. E. HAFEZ & G. PINCUS. 1956a. *Proc. 3rd Internat. Congr. Animal Reprod.* Cambridge. : 48-50.
- FRIDHANDLER, L., E. S. E. HAFEZ & G. PINCUS. 1956b. Respiratory metabolism of mammalian eggs. *Proc. Soc. Exptl. Biol. Med.* **92**: 127-129.
- FRIDHANDLER, L., E. S. E. HAFEZ & G. PINCUS. 1957. Developmental changes in the respiratory activity of rabbit ova. *Exptl. Cell Research.* **13**: 132-139.
- GALTSOFF, P. S. & E. GALTSOFF. 1953. Destruction, survival, and disorganized growth of embryonic organs and tissues of *Opsanus tau* after homoplastic transplantation of whole embryos in the adult toadfish. *Anat. Record.* **117**: 601.
- GEY, G. O., G. E. S. JONES & L. M. HELLMAN. 1938. The production of a gonadotrophic substance (prolan) by placental cells in tissue culture. *Science.* **88**: 306-307.
- GOMORI, G. 1952. *Microscopic Histochemistry.* Univ. Chicago Press. Chicago, Ill.
- GREENWALD, G. S. 1957. Interruption of pregnancy in the rabbit by the administration of estrogen. *J. Exptl. Zool.* **135**: 461-482.
- HERTIG, A. T. 1935. Angiogenesis in the early human chorion and in the primary placenta of the macaque monkey. *Contrib. to Embryol. Carnegie Inst. Wash.* **25**: 37-81.
- HERTIG, A. T. & H. W. EDMONDS. 1940. Genesis of hydatidiform mole. *A. M. A. Arch. Pathol.* **30**: 260-291.
- HERTIG, A. T. & J. ROCK. 1941. Two human ova of the pre-villous stage, having an ovulation age of about eleven and twelve days respectively. *Contrib. to Embryol. Carnegie Inst. Wash.* **29**: 127-156.
- HILL, J. P. 1910. (In 1911 vol.) The early development of the Marsupialia with special reference to the native cat (*Dasyurus* v.) *Quart. J. Microscop. Sci.* **56**: 1-134.
- HOLTGRETER, J. 1944. Experimental studies on the development of the pronephros. *Rev. can. biol.* **3**: 220-250.
- HUBRECHT, A. A. W. 1888. Keimblätterbildung und Placentation des Igels. *Anat. Anz.* **3**: 510-515.
- HUBRECHT, A. A. W. 1889. (In 1890 vol.) Studies in mammalian embryology. I. The placentation of *Erinaceus europaeus*, with remarks on the phylogeny of the placenta. *Quart. J. Microscop. Sci.* **30**: 283-404.
- JONES, G. E. S., G. O. GEY & M. K. GEY. 1943. Hormone production by placental cells maintained in continuous culture. *Bull. Johns Hopkins Hosp.* **72**: 26-38.
- KREBS, E. T., JR., E. T. KREBS, SR. & H. H. BEARD. 1950. The unitarian or trophoblastic thesis of cancer. *Med. Record.* **163**: 149-174.
- LUTWAK-MANN, C. 1954. The occurrence of carbonic anhydrase in the rabbit uterus. *J. Endocrinol.* **11**: xi.
- LUTWAK-MANN, C. 1955. Carbonic anhydrase in the female reproductive tract. Occurrence, distribution and hormonal dependence. *J. Endocrinol.* **13**: 26-38.
- LUTWAK-MANN, C. & R. L. W. AVERILL. 1954. Carbonic anhydrase in the uterus and Fallopian tubes of the ewe. *J. Endocrinol.* **11**: xii.
- LUTWAK-MANN, C. & H. LASER. 1954. Bicarbonate content of the blastocyst fluid and carbonic anhydrase in the pregnant rabbit uterus. *Nature.* **173**: 268-270.
- MAXIMOW, A. 1925. Tissue cultures of young mammalian embryos. *Contrib. to Embryol. Carnegie Inst. Wash.* **16**: 47-113. (Plus 13 plates.).
- MCCRADY, E., JR. 1938. The embryology of the opossum. *Am. Anat. Mem. No. 18.* Wistar Inst. Anat. Biol. Philadelphia, Pa.
- MCCRADY, E., JR. 1944. The evolution and significance of the germ layers. *J. Tenn. Acad. Sci.* **19**: 240-251.
- MOSSMAN, H. W. 1937. Comparative morphogenesis of the fetal membranes and accessory uterine structures. *Contrib. to Embryol. Carnegie Inst. Wash.* **26**: 129-246.
- PAPACONSTANTINO, J. & S. P. COLOWICK. In press.
- SCHOENFELD, H. 1903. Contribution à l'étude de la fixation de l'oeuf des Mammifères dans la cavité utérine, et dans premiers stades de la placentation. *Arch. Biol. Paris.* **19**: 701-830.

- SMALBRAAK, J. 1957. Trophoblastic Growths. Elsevier. Amsterdam, Holland, London, England, New York, N. Y., Princeton, N. J.
- STEINBERG, M. S. 1958. On the chemical bonds between animal cells. A mechanism for type-specific association. *Am. Naturalist*. **92**: 65-81.
- SUMNER, J. B. & G. F. SOMERS. 1953. Chemistry and Methods of Enzymes. 3rd ed. Academic Press. New York, N. Y.
- VON BRAND, T. 1946. Anaerobiosis in Invertebrates. Biodynamica. Normandy, Mo.
- WARBURG, O. 1956. On the origin of cancer cells. *Science*. **123**: 309-314.
- WEINHOUSE, S., O. WARBURG, D. BURK & A. L. SCHADE. 1956. On respiratory impairment in cancer cells. *Science*. **123**: 267-272.
- WISLOCKI, G. B. & G. L. STREETER. 1938. On the placentation of the macaque (*Macaca mulatta*), from the time of implantation until the formation of the definitive placenta. *Contrib. to Embryol. Carnegie Inst. Wash.* **27**: 1-66.
- WOGLOM, W. H. 1947. General review of cancer therapy. *In* Approaches to Tumor Chemotherapy. F. R. Moulton, Ed. Am. Assoc. Advancement Sci. Washington, D. C.

HOMOPLASTIC IMPLANTATION OF TOADFISH (*OPSANUS TAU*) EMBRYOS IN THE PERITONEAL CAVITY OF AN ADULT FISH

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Many difficulties and frequent failures in inducing the transplanted tissues and organs of animals to survive permanently and to grow led to the idea of using embryonic rather than adult material. The description of the results of many experiments with invertebrate and vertebrate animals are summarized by May (1932); additional and more recent data may be found in the books on experimental embryology by Weiss (1939), and by Willier *et al.* (1955).

The embryonal hypothesis of the origin of carcinomas, popular at the turn of the Nineteenth Century, also stimulated considerable interest in experiments involving the transplantation of eggs and embryos in the tissue and organs of various vertebrates. Of particular theoretical interest were the results of experiments with Amphibia (both Urodela and Anura) in which eggs at early stages of development and embryos were transplanted into the abdominal cavities of adults. It was claimed that under certain conditions the implants gave origin to malignant tumors (sarcoma) which in 5 to 6 months killed the host (Belogolowy, 1918; Dürken, 1926). Certain tissues developed from the transplanted embryos in more or less typical manner and produced teratomas and embryomas (Bosaeus, 1926). On the other hand, the embryos of toads and frogs (*Bufo vulgaris* and *Bombinator igneus*) implanted in the abdominal cavity underwent gradual dedifferentiation and formed large masses of simplified cells (Weber, 1920). Similar results were obtained with toadfish embryos (Galtsoff and Galtsoff, 1953).

In the other type of experiments, the dissociated cells of chick embryos were injected intravenously into adult birds (Weiss and Andres, 1952) and into the embryos (Andres, 1953). It was shown by these studies that various embryonic cells dispersed by the blood stream throughout the adult organism invaded its tissues, proliferated profusely, and colonized the locations that offered appropriate specific conditions for a cell of particular type. In a nonmatching environment a roaming cell of a given strain failed to proliferate and did not express its developmental potency.

Dissociated cells injected into the embryos settled on embryonic membranes only and produced teratomas of variable complexities. The experiments clearly indicate the importance of the specific environment in determining the fate of the roaming embryonic cells.

The purpose of the present experiments was twofold: (1) to determine the effects of grafting on further development of the fish embryo, and (2) to study the reaction of the host to embryonic organs and tissues introduced into the peritoneal cavity.

The use of toadfish for transplantation experiments has several advan-

tages. The fish is of convenient size, measuring from 6 to 9 inches in length, is very common in shallow waters of the Atlantic Coast, and can be captured easily. Keeping the toadfish in captivity presents no difficulty. They feed on small live fish, but can be coached to take dog-food pellets. Furthermore, the fish is extraordinarily hardy and apparently is immune to bacterial infections. Its skin is very tough, but has no scales. The eggs are large, about 6 mm. in diameter. They are yellowish, transparent, and are attached to submerged objects from which they can be removed without injury by using a sharp knife.

The following method of implantation was used: eggs removed from their substratum were washed several times in sterile sea water, placed in a Syracuse dish, and examined under the dissecting microscope to ascertain the state of their development. Under aseptic conditions, the embryos varying in length from 5 to 9 mm. were removed from the egg by sharp sterile needles and separated from their large yolk sacs. After repeated rinsing in sterile sea water the embryos with the remaining membranes were introduced through small abdominal incision into the peritoneal cavity. The incision was closed by several stitches and the fish returned immediately to running sea water. During the operation the fish was kept under full narcosis induced by a 10 per cent solution of urethane in sea water. During the period of recovery, which lasted for several hours, the operated specimen floated with its belly up. This position facilitated the settling of the injected embryos on the surface of a very large liver. The embryos used for implantation were at the advanced stages of development and varied from 5 to 9 mm. in length; they had well-formed eyes, neural plates, notochord, intestine, muscle somites, and blood vessels. Observation of heartbeat served as a check that the embryos were alive.

At intervals varying from 2 to 55 days the fish were dissected and their tissues preserved for sectioning. Most of the implants were found on the surface of the liver, but a few were affixed to the abdominal wall and mesentery.

Areas of discolored tissue marked the location of an implant on the surface of the liver. If the embryos attached themselves to the abdominal wall or mesentery, they formed oval-shaped pedunculated cysts freely suspended in the peritoneal cavity. In a short time all implants became well vascularized and were supplied by blood from the host.

Forty-eight hours after injection the embryos were found firmly attached to the surface of the liver. Frequently they were located in the fissures of the liver lobes. The position of the implant was marked by an irregularly shaped, semitransparent white area varying in length from a few millimeters to about two cm. Sections across these early stages of implantation show the embryo resting in a slight depression formed by the receding organ of the host (FIGURE 1). Embryonic skin, clearly recognizable by the presence of large mucous cells, adheres closely to the liver, the adjacent portions of which do not show any pathological reaction.

The eyes of the implants with the intact lenses are normal and the midbrain retains the typical appearance, although its cells begin to disintegrate.

Close contact between the skin of the implant and the liver of the host is clearly seen on the section through the head of another 48-hour graft (FIGURE 2). No pathological changes are noticeable either in the liver or in the skin of the embryo. A certain degree of disintegration appears, however, in a 2-day-old implant that settled in the fissure between the two lobes of the liver of the same toadfish (FIGURE 3). The notochord and the somites seen in the lower portion of the implant retained their normal position, while considerable disorientation took place in the upper section of the graft where:

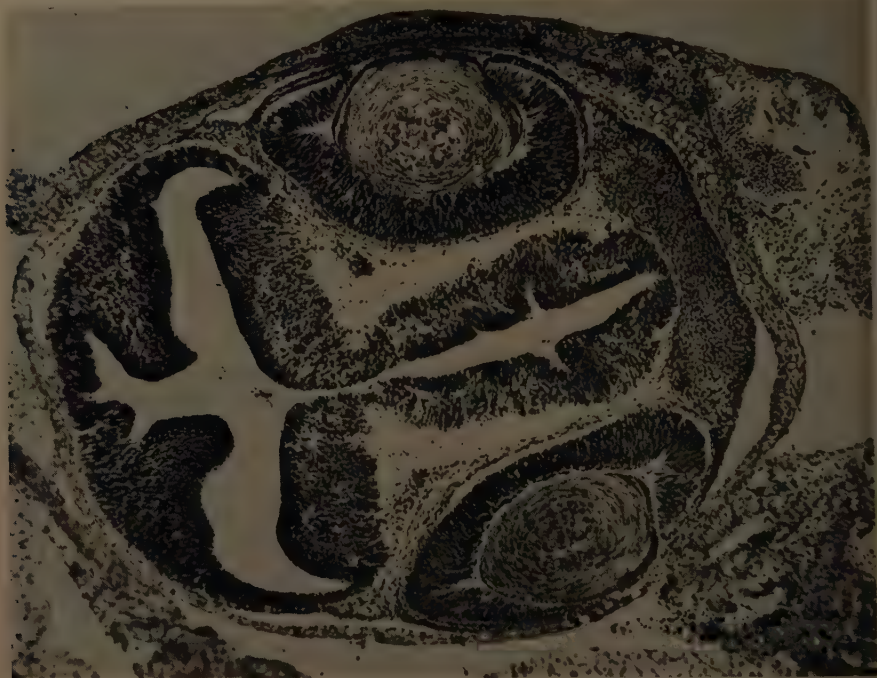


FIGURE 1. Embryo 5 mm. long, 48 hours after the injection into the peritoneal cavity of an adult fish. Embryo adhered to the surface of the liver. $\times 80$.

the irregular pieces of cartilagenous tissue are surrounded by a bulky layer of mesenchyme.

As the implant continues to grow the arrangement of the surviving organs becomes so distorted that the former organization of the embryo is completely lost. The resulting mass of tissue assumes an irregular shape in which no traces of former relation of organs to each other can be found, though some of them can be still identified by their histological structure. A section across a 14-day-old implant encapsulated on the surface of the liver (FIGURE 4) illustrates this stage. At dissection, this implant appeared as a round white cyst slightly protruding above the surface of the liver. The latter was found partially adhering to the abdominal wall. In spite of the considerable increase in bulk, the implant was sharply separated from the

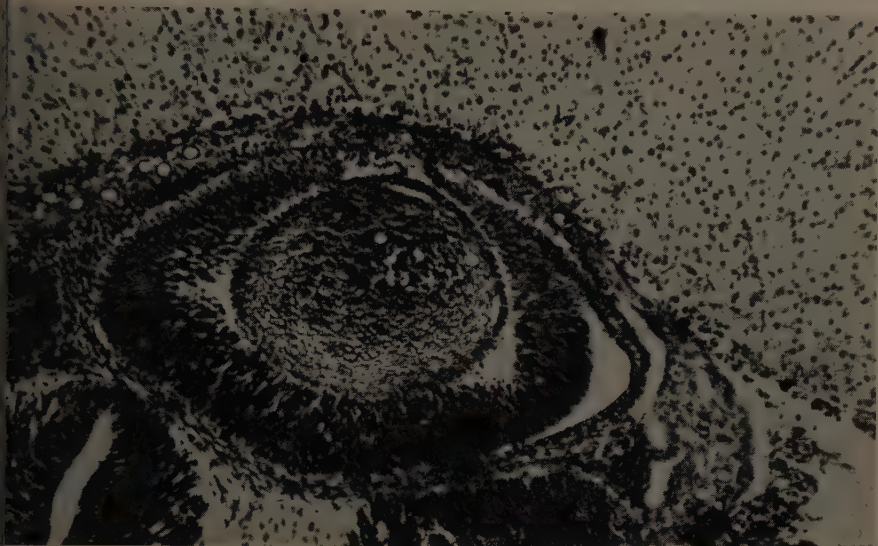


FIGURE 2. Close contact between the liver and the skin of an embryo 48 hours after injection. Large mucus cells of the skin are clearly visible in the upper left corner. No pathological reaction in the liver cells. $\times 135$.



FIGURE 3. Longitudinal section through the trunk of an embryo settled in the fissure between the lobes of the liver 48 hours after injection. Chorda and somites retain their normal position (lower half). Disorganization of the organs is noticeable in the upper half of the implant. $\times 60$.

underlying tissue of the host. Its mass consisted of a prolifically growing mesenchyme with several pieces of notochord recognizable by its typical cartilagenous cells. The twisted and misshapen appearance of notochord had but little resemblance to the original skeletal element of the embryo. The brain and all the organs of endodermic origin were found at various stages of destruction by the invasion of blood cells.

The process of disorganization in some of the implants occurs more rapidly than in others. For instance, the notochords of some 6-day-old implants were found partially destroyed and the cells of nervous system and intestines cytolized; in other implants of the same age the general disintegration of

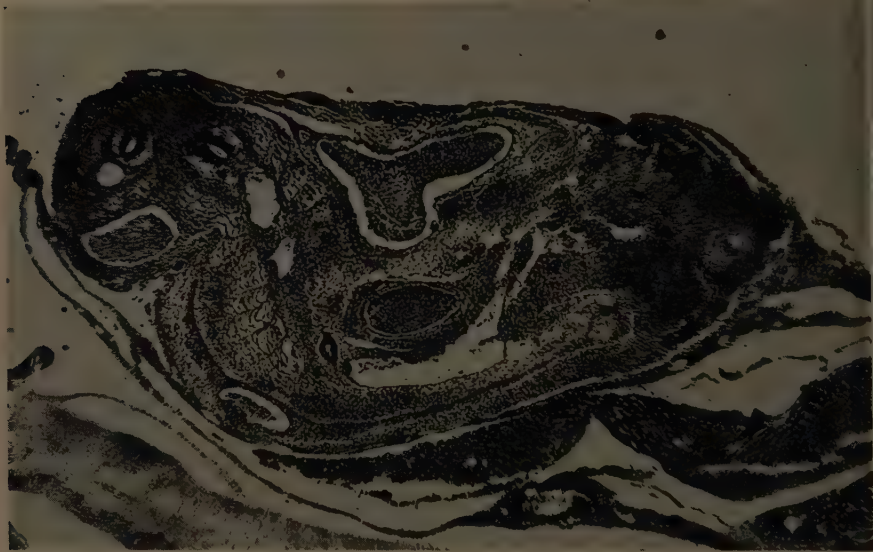


FIGURE 4. Disarrangement of organs and partial destruction of some of them in a 14-day-old implant settled on the surface of the liver. $\times 60$.

organs was less advanced. Some of the implants were not encapsulated; the embryonic connective tissue cells invaded and penetrated deep into the liver of the host (FIGURE 5).

Of particular interest are the old implants in which continuous growth of the notochord resulted in the formation of irregular and sometimes grotesque structures that apparently thrived in the liver. The sections through the 38-day-old implant show greatly enlarged and twisted notochord (FIGURE 6) that in places resembles a vertebra with the ribs attached at both sides (FIGURE 7). The 55-day-old implant (the oldest in the series of the experiments) consisted entirely of connective tissue (FIGURE 8) that had penetrated into the liver and destroyed considerable portions of it. Scattered islands of dying liver cells can still be seen in this implant.

The fate of the notochord varies. In some of the implants the notochord is broken into small pieces that undergo disintegration, as can be seen in

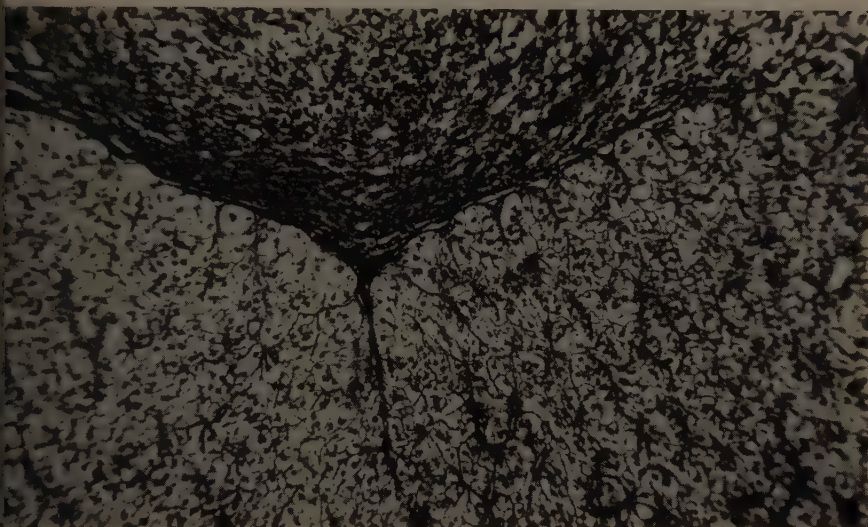


FIGURE 5. Invasion of the liver of the host by the fibroblasts growing from a 6-day-old implant settled on the surface of the liver. $\times 155$.

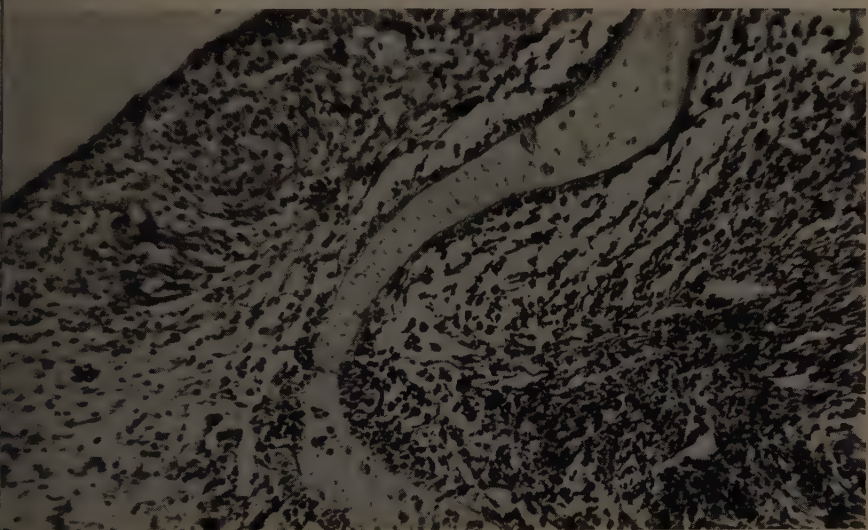


FIGURE 6. Persistent but irregular growth of the notochord in a 38-day-old implant on the surface of the liver. Note the profuse growth of connective tissue. $\times 180$.

the structure shown in FIGURE 9. Loose connective cells and blood cells penetrate through a partially damaged outer membrane and destroy the notochord. Some of the cartilagenous cells are cytolized and are being replaced by fibroblasts. In other cases (FIGURE 10) healthy and vigorous pieces of the notochord retain their typical structure. Cartilagenous cells



FIGURE 7. Growth of the notochord in a 38-day-old implant in the liver. The structure assumes a certain resemblance to vertebra and ribs. $\times 150$.

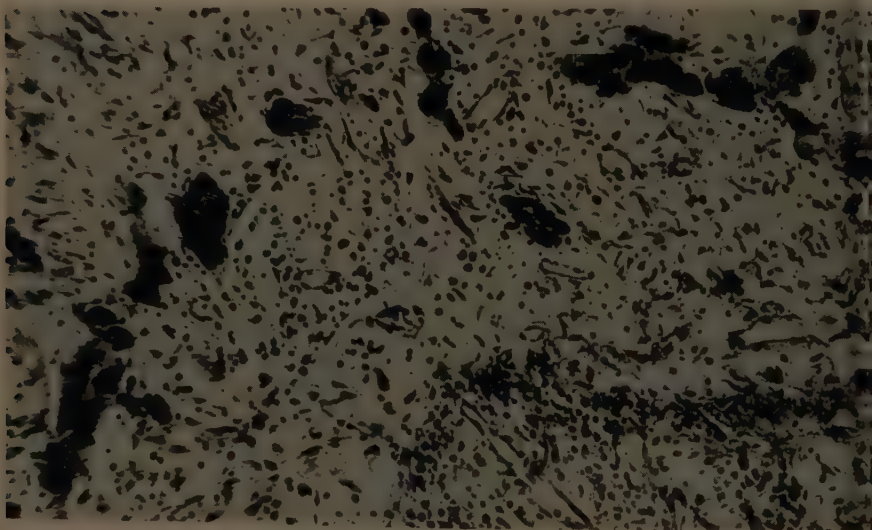


FIGURE 8. Destruction of a portion of liver by the connective tissue invaded from an implant. Dying liver cells are shown as black masses of irregular shape; 17-day-old implant in liver. $\times 200$.

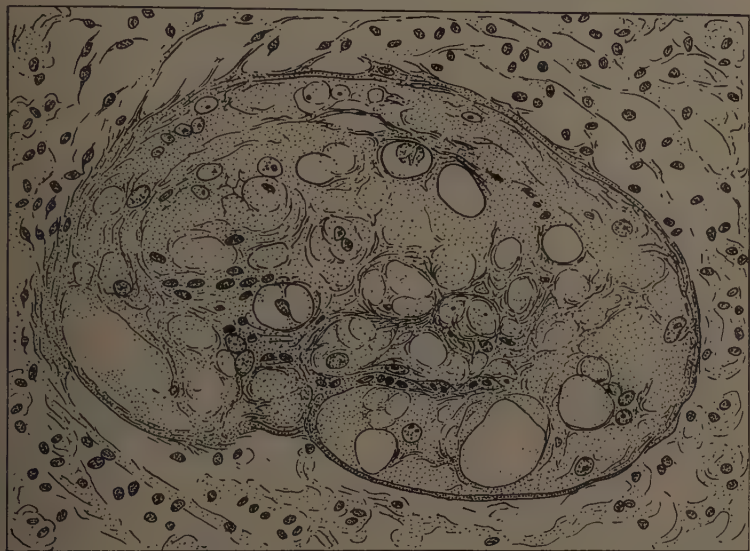


FIGURE 9. Piece of notochord in the 17-day-old liver implant. Oil immersion. Camera lucida drawing. $\times 625$.

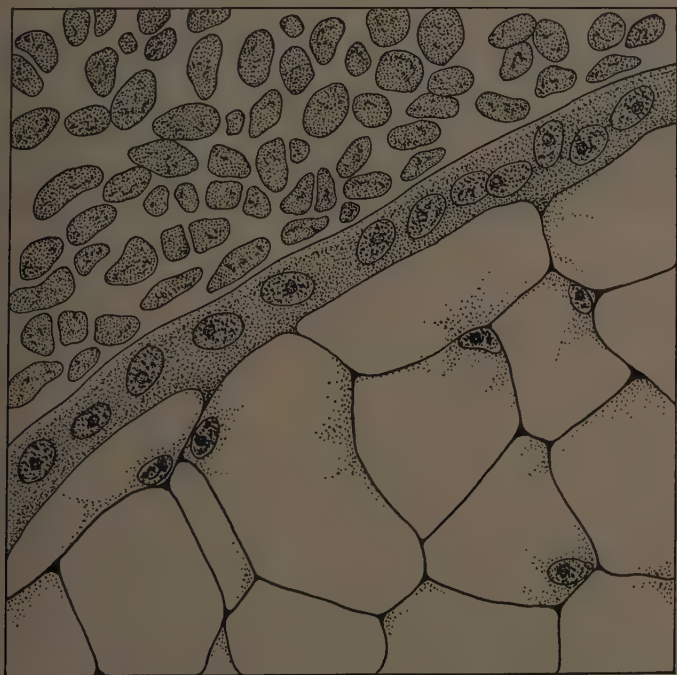


FIGURE 10. Piece of healthy notochord growing in a 17-day-old liver implant. Camera lucida drawing. Oil immersion. $\times 625$.

surrounded by chorda epithelium show no signs of disintegration. Under favorable conditions the notochord probably persists for a long time.

The implants that attached themselves to the abdominal wall or to the mesentery formed cysts freely suspended in the peritoneal cavity. Their long peduncles contained blood vessels connecting them to the circulatory system of the host. The core of the cysts was made of mesenchyme cells containing scattered pieces of notochord. The presence of the notochord in these cysts served as evidence of their embryonic origin. Frequently the connective tissue cells were oriented concentrically around wide and empty canals (FIGURE 11), the origin and function of which are not known.

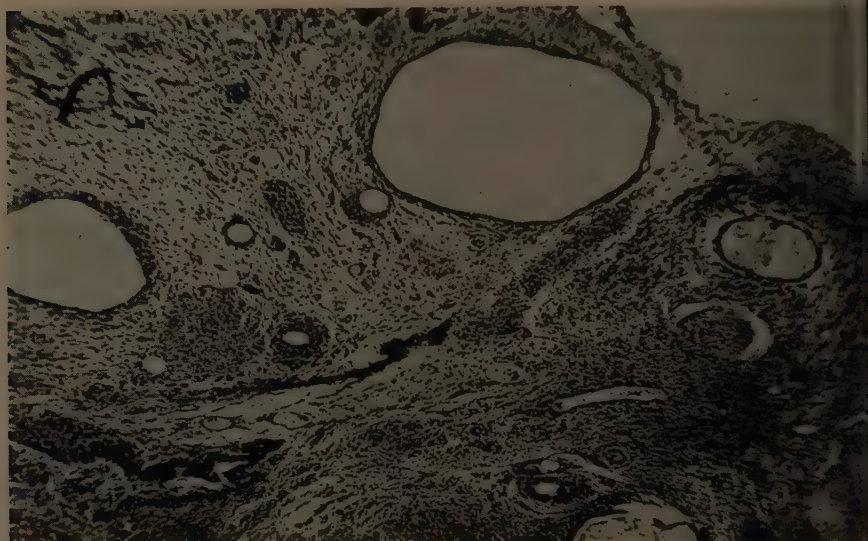


FIGURE 11. Structure of a cyst suspended from the abdominal wall. Note the large canals in the mass of mesenchyme tissue; 55-day-old implant in the liver. $\times 80$.

Because of the presence of notochords the cysts may be considered as teratomas. Their presence either as liver implants or as cysts had no visible adverse effects on fish, which were vigorous, had good appetite, and behaved normally.

Because of the technical difficulties in maintaining marine aquariums through the winter, the experiments were discontinued on the fifty-fifth day after implantation of the embryos, and the eventual fate of tumors and their effect on toadfish are not known. Histological studies of the material failed to find any metastases and did not indicate the malignancy of the tumors.

The following inference can be drawn from the observations: homoplastic implantation of toadfish embryos immediately suppresses the organized development of the embryo. The tissues of various organs of the embryo are thrown into unrestricted competition in which the nervous system and the endodermic elements are rapidly destroyed. The notochord continues

grow in an irregular and frequently grotesque manner, forming structures remotely resembling the skeletal elements of the fish. In the struggle for existence the connective tissue survives and continues to grow, invading and destroying the underlying tissues of the host.

References

- ANDRES, G. 1953. Experiments on the fate of dissociated embryonic cells (chick) disseminated by the vascular route. II. Teratomas. *J. Exptl. Zool.* **122**: 507-540.
- BELOGOLOWY, G. 1918. Die Einwirkung parasitären Lebens auf das sich entwickelnde Amphibienei (den "Laichball"). *Arch. Entwicklungsmech. Organ.* **43**: 556-681.
- OSAEUS, W. 1926. Beiträge zur Kenntniss der Genese der Ovarienembryome. *Almqvist und Bocktryckeri, Eds.* 303 pp. Uppsala, Sweden.
- ÜRKEN, B. 1926. Das Verhalten embryonaler Zellen im Interplantat. *Arch. Entwicklungsmech. Organ.* **107**: 727-828.
- GALTISOFF, P. S. & E. GALTISOFF. 1953. Destruction, survival and disorganized growth of embryonic organs and tissues of *Opsanus tau* after homoplastic transplantation of whole embryos in the adult toadfish. *Anat. Record.* **117**: 601.
- LAY, R. M. 1932. *La Transplantation Animale.* 352 pp. Gauthier-Villars. Paris, France.
- EBER, A. 1920. Greffes d'oeufs de Batraciens Urodèles dans la cavité péritonéale d'adultes de la même espèce. *Compt. rend. soc. biol.* **83**: 749-751.
- EBER, A. 1920. Évolution prolongée de larves d'un Batracien Anoure, *Bombinator igneus*, dans le sac lymphatique dorsal d'adultes de la même espèce. *Compt. rend. soc. biol.* **83**: 1058-1060.
- WEISS, P. 1939. *Principles of Development.* 601 pp. Holt. New York, N. Y.
- WEISS, P. & G. ANDRES. 1952. Experiments on the fate of dissociated embryonic cells (chick) disseminated by the vascular route. *J. Exptl. Zool.* **121**: 449-488.
- WILLIERS, B. H., P. A. WEISS & V. HAMBURGER. 1955. *Analysis of Development.* 735 pp. Saunders. Philadelphia, Pa.

TROPHOBLAST: PROBLEMS OF INVASION AND TRANSPORT

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The degree to which trophoblast can penetrate and grow within maternal tissues is determined, not only by the erosive and proliferative proclivities of the trophoblast cells, but by receptive and defensive mechanisms in the endometrium and in the maternal vascular system. This paper deals with certain aspects of trophoblastic activity encountered in two experiments recently conducted in our laboratory. However, these efforts and those of many others, only a few of which can be mentioned in this brief review, have by no means solved the problems of invasion and transport of trophoblast.

Early workers believed that the trophoblast eroded the surface epithelium of the endometrium and elicited the edema, hemorrhage, and decidual reactions so characteristic of mammalian implantation. Blandau¹ showed that all of these endometrial reactions could be duplicated by introducing small inert particles into the endometrial cavity early in pregnancy. This work raised the serious question of whether the trophoblast played any essential role in the early phases of implantation.

Fawcett *et al.*² transferred early blastocysts into the anterior chamber of the eye in mice and noted that hemorrhage and edema of the iris occurred in 1 to 2 days, but attachment did not occur until the blastocyst was 7 rather than the normal 5, days of age. When they transplanted small bits of 9-day-old trophoblast, however, these authors noted almost immediate hemorrhage and edema, and attachment occurred in 1 day. Under these conditions the capillaries of the iris were eroded, but they did not proliferate to form placental sinusoids. No decidual reaction was elicited, and the trophoblast penetrated only 1 or 2 cell layers of the iris. Fawcett *et al.* logically concluded that both the endometrium and the trophoblast played essential roles in the process of implantation. Their experiments suggested very strongly, moreover, that the stage of trophoblastic maturation might be an important factor in determining when implantation could occur.

This background suggested the following hypothesis. Since the receptive and defensive mechanisms of the endometrium and the erosive-proliferative mechanisms of the trophoblast are undergoing rapid maturational changes at the time of implantation, experimental alteration of the stage of maturation of the trophoblast in relation to the stage of development of the endometrium should alter significantly the net invasiveness or implantability of the trophoblast. Our method was to transfer fertilized, genetically marked ova 1 to 5 days of age into the reproductive tracts of recipient rats on each of the first 7 days of pregnancy. If the ovum implanted successfully the resulting embryo could be distinguished by its color from the control embryos occupying the same uterine horn. The percentage of transferred ova that survived to term embryos was taken as a measure of the net implantation

bility of trophoblast of that particular stage of maturation at that particular phase of endometrial development.

An example of the results obtained from one such set of transfer experiments is given in FIGURE 1. Each black dot represents the transfer of approximately 5 ova (obtained from the oviducts of pigmented donor rats in the first day after mating) into the oviducts of albino recipient rats on various days of the estrous cycle and early pregnancy. The position of the dot on the ordinate axis indicates the percentage of transferred ova that implanted normally and survived to term. Preliminary experiments had

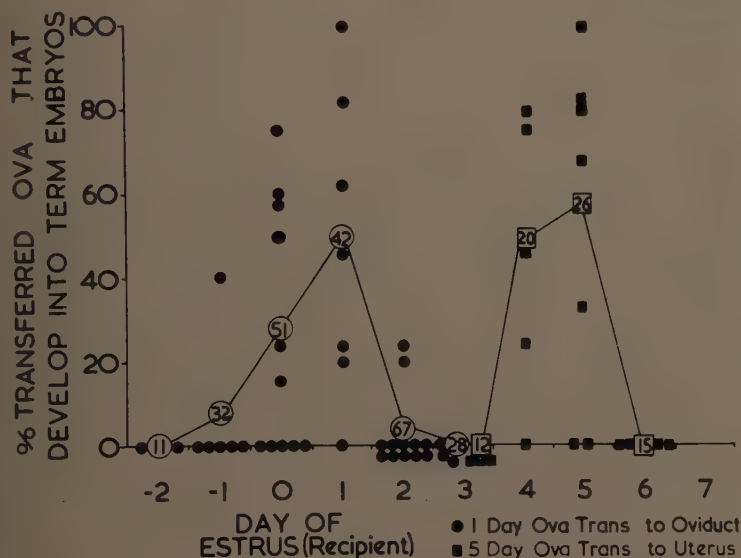


FIGURE 1. The percentages of 1-day-old and 5-day-old rat ova that developed into term embryos following transfer into the oviducts and into the uterine horns of recipient rats on various days of the estrous cycle and early pregnancy. The enclosed figures indicate the total number of ova transferred on a given day.

shown that ova transferred to the oviducts entered the uterus 4 days after transfer regardless of what day of the cycle the transfer was performed. Therefore, these 1-day ova transferred to the oviduct were 5 days old when they reached the endometrial cavity. For this reason the data on transfers of 1-day ova into the oviducts can be summated with those of 5-day ova transferred into the uterus (black squares in FIGURE 1).

Such a summation has been made in FIGURE 2, in which it can be seen that more than half of the ova survived transfer in the control group. Of 11 5-day ova that entered the uterus on the fourth day, one third survived, but of 82 5-day ova that entered the uterus on the sixth day, only 2 survived. Similar experiments were carried out with ova at later stages of development, and in each case the survival curves were skewed in the same direction, that is, a precipitous fall in implantations occurred when the ova entered the endometrial cavity 1 or more days ahead of their own stage of development.

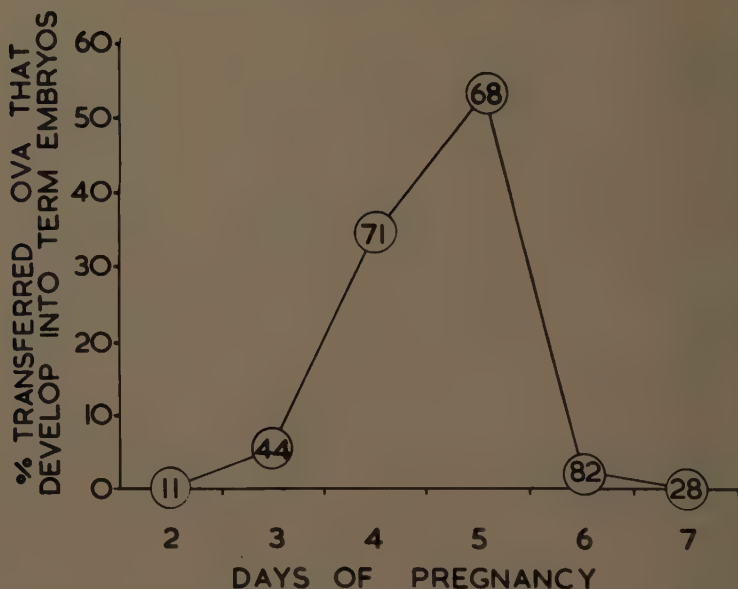


FIGURE 2. The data of FIGURE 1 summated in terms of the percentages of 5-day ova that develop into term embryos on entering the endometrial cavity on a given day of pregnancy.

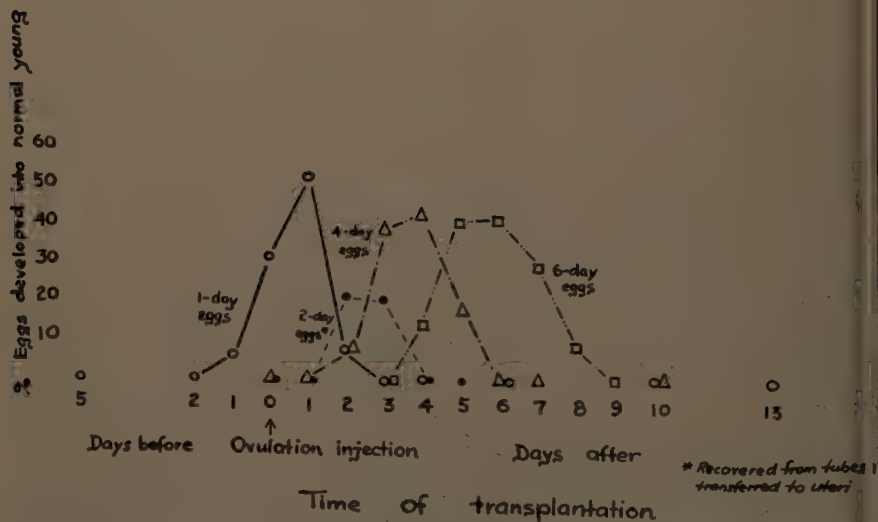


FIGURE 3. Development of transferred ova or blastocysts in relation to ovulation time of recipients. Data and figure by Chang,³ reproduced by permission from *The Journal of Experimental Zoology*.

In many cases survival was actually better when the ova were 1 or 2 days ahead of the endometrial development than when the ova and the endometrium were at the same stage of development. Whether this finding will prove to be statistically valid, however, depends on work still in progress.

Chang³ transferred ova into the reproductive tracts of pseudopregnant rabbits and concluded that, to be successful, ova must be transferred within 1 or 2 days of their own stage of development. Inspection of Chang's survival curves (FIGURE 3), however, discloses a marked tendency toward the same kind of skewness we have found in the case of the rat.

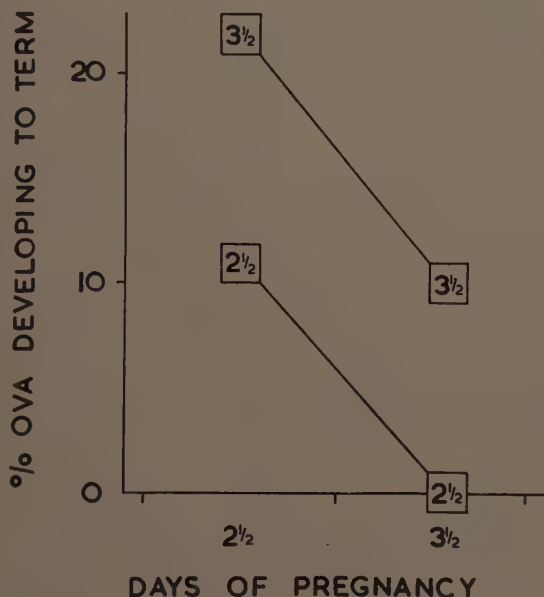


FIGURE 4. The percentages of $2\frac{1}{2}$ - and $3\frac{1}{2}$ -day ova that developed into term embryos following transfer into the uterine horns of recipient mice $2\frac{1}{2}$ and $3\frac{1}{2}$ days after the onset of pregnancy. Data from McLaren and Michie,⁴ reproduced by permission from *The Journal of Experimental Biology*.

McLaren and Michie⁴ transferred $2\frac{1}{2}$ - and $3\frac{1}{2}$ -day-old ova into the uterine cavities of mice $2\frac{1}{2}$ - and $3\frac{1}{2}$ -days pregnant. Here again (FIGURE 4) survival was significantly better when the ova were 1 day advanced in maturation than when ova and endometrium were at the same stage of development. When the ova were 1 day less developed than the endometrium none survived.

Thus it would appear that, in the 3 species studied so far, trophoblastic maturation is a very critical factor in the implantation mechanism. In order successfully to penetrate the endometrium and survive, the trophoblast must first have matured to or beyond the corresponding stage of endometrial development. Conversely stated, in terms of receptive-defensive mechanisms, if the endometrial development is 1-day more advanced than that of the trophoblast, implantation is inhibited.

A second series of experiments conducted recently in our laboratory⁵ was stimulated in part by the work of Hertig and Edmonds,⁶ who reported that early fetal death causes deficient vascularization of placental villi followed by trophoblastic proliferation and hydatid degeneration. The experiments also were stimulated in part by the work of Huggett and Pritchard,⁷ who noted that when rat embryos were removed or destroyed after midpregnancy the trophoblast proliferated excessively before undergoing necrosis. Selye *et al.*⁸ showed that when both ovaries were removed in the rat early in preg-

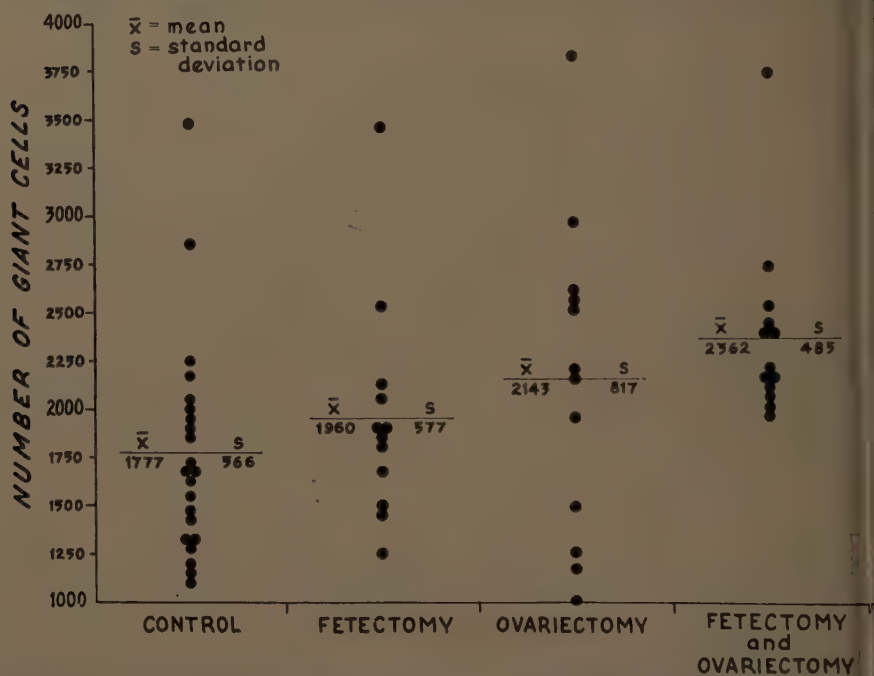


FIGURE 5. Numbers of trophoblast giant cells counted in one fiftieth of each of 59 rat gestation sacs, as affected by fetectomy, ovariectomy, and ovariectomy plus fetectomy. Symbols: $\bar{x}$ = mean, s = standard deviation.

nancy, before the chorioallantoic placenta was formed, the pregnancy was absorbed or aborted, but that when the embryos as well as the ovaries were removed the placentas usually survived. Fawcett⁹ noted that, although no fetal elements survived when mouse ova were transplanted beneath the kidney capsule, the trophoblast grew excessively.

These results suggested the following hypothesis: absence of the fetus may favor trophoblastic proliferation, withdrawal of hormonal support may diminish endometrial resistance, and these factors working alone or in concert should alter trophoblastic growth and invasiveness. To test this hypothesis, 17 adult rats were operated upon on the twelfth day of pregnancy. This time was selected because Bridgman¹⁰ had reported that a burst of mitotic activity occurs in the reticular cytotrophoblast on the twelfth

ay. The fetuses were removed from 12 gestation sacs (fetectomy group) and sham operations were performed on 22 gestation sacs (control group) of 8 animals. In another group of 9 animals both ovaries were removed, fetuses were removed from 22 sacs (ovariectomy-fetectomy group), and sham operations were performed on 19 sacs (ovariectomy group).

Two days later the uteri were removed, and the gestation sacs were serially sectioned, each fiftieth section being mounted and stained. The syncytial giant cells were counted, and the volume of the cytotrophoblast was esti-

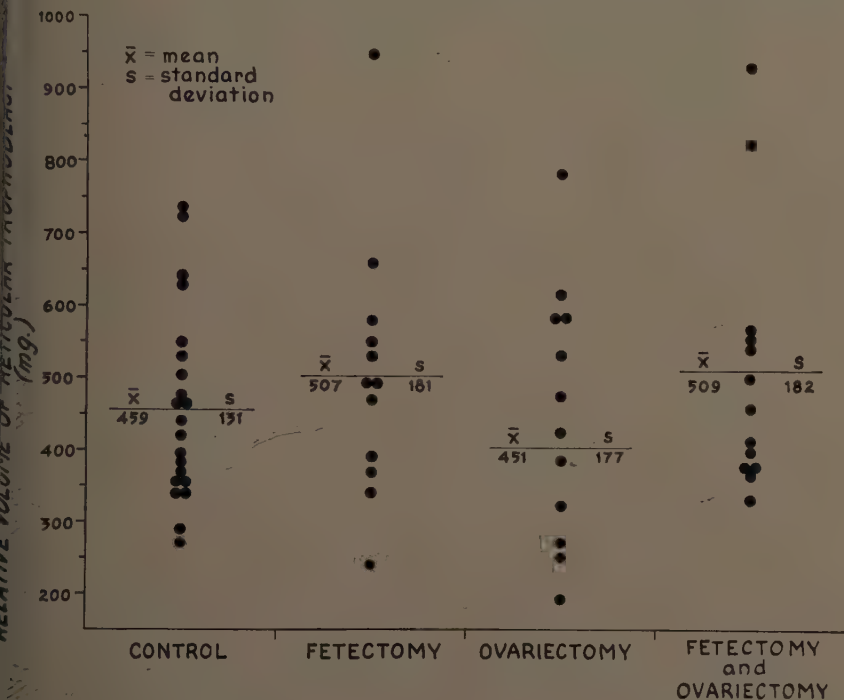


FIGURE 6. Relative volumes of the reticular trophoblast zone in one fiftieth of each of the same 59 gestation sacs shown in FIGURE 5.

ated from projected silhouettes, which were traced on paper, cut out, and weighed.

The relative number of trophoblast giant cells in the four groups is shown in FIGURE 5, and the volume of reticular trophoblast in FIGURE 6. These data were summated factorially in such a way that the information contained in each group could be simultaneously compared with the others. This method is more efficient than comparing the differences in means of each experimental group with that of the control group.

Fetectomy caused an insignificant increase in numbers of giant cells ($p = 0.23$) and volume of cytotrophoblast ($p = 0.27$), when compared with gestation sacs containing embryos. Ovariectomy was followed by a significant rise in the giant-cell count ($p = 0.02$), but no increase in cytotrophoblast

($p = 0.95$). A test showed that no interaction existed between the effect of fetectomy and ovariectomy, so that what appeared to be a potentiating effect of fetectomy on the increase in numbers of giant cells caused by ovariectomy was not significant.

Alternative hypotheses suggest themselves to explain the increase in number of giant cells following ovariectomy. First, it is possible that the number of giant cells increases as a result of a stepped-up rate of differentiation from the cytotrophoblast. A second possibility is that the apparent increase in the number of giant cells is, in reality, a failure of involution of the giant-cell zone, a process that normally occurs between the twelfth and fourteenth days of pregnancy. Finally, it is possible that some mechanical change in the neighborhood of the decidua, particularly the decidua capsularis, might have made giant cells (previously too stretched out to be counted) more easily recognizable following ovariectomy with its consequent shrinkage of the gestation sac. Of these three possibilities it is believed that the first is the most likely, but unfortunately our methods did not permit positive evaluation of this important point.

The wide range of observations in each group point to the high degree of variability of all the aspects of trophoblastic growth studied quantitatively in this experiment. In each group the individual giant-cell counts and reticular volumes varied as much from one gestation sac to another in the same animal as they did from one animal to another. This variability therefore seems to be inherent to each gestation sac and does not seem to be related to the amount of available hormones, uterine accommodation, or minor variations in experimental technique.

Although the trophoblastic giant cells appear to be chiefly responsible for erosion of the decidua, it is the smaller, reticular cytotrophoblast cells that invade the maternal vascular tree. Neither quantitative nor qualitative observations in our study suggest that ovariectomy or fetectomy increases the invasiveness of these cells. It is interesting that McKay *et al.*¹¹ have found that anoxia causes increased proliferation, although apparently not increased invasiveness of cytotrophoblast in the human placenta. Anoxia appeared to be very detrimental to the syncytial giant cells, however, in such a manner that no relationship would appear to exist between the effect of ovariectomy in the rat and anoxia in the human placenta.

In summary, it must be repeated that the two series of experiments reviewed in this report have by no means solved the problem of how the trophoblast invades tissue, or how this invasion is usually so well controlled by the maternal organism. However, our work supplements that of others in emphasizing points worthy of further study. The invasiveness of primitive trophoblast seems to depend upon its state of maturation, perhaps because the elaboration and secretion of complex proteolytic enzymes require a rather advanced state of cellular differentiation not yet developed in the early blastocyst. This process, although it favors normal trophoblastic invasion, may have nothing in common with the apparent reversal in differentiation that accompanies the so-called malignant degeneration preceding the uncontrolled invasiveness of chorionepithelioma.

The facts that inert particles incite implantation reactions in the endometrium, but not elsewhere, and that trophoblast fails to incite complete implantation reactions except in the endometrium suggest that the endometrium actively accepts or receives the trophoblast. Trophoblast does not penetrate deeply into tissues other than the endometrium and uterine blood vessels, and this would suggest again that the control of trophoblastic penetration may not depend so much upon the strength of tissue defenses as upon rapidly changing and highly specialized receptive functions within the endometrium. These functions are certainly influenced by steroid hormones, but the relative roles played by the ovarian and placental hormones in altering endometrial receptiveness and resistance are far from known. The evidence that abrupt withdrawal of ovarian support causes an increase in number of giant cells advances our knowledge a little, although this change appeared to favor differentiation rather than invasion of the trophoblast. What is needed is an exhaustive study of the quantitative response of trophoblast to controlled levels of estrogen and progesterone at various stages of pregnancy. Although our particular experiment failed to show that the fetus had much effect on trophoblastic activity, evidence from other sources strongly suggests that this is another important variable that must be considered in this fascinatingly elusive problem.

References

1. BLANDAU, R. J. 1949. Embryo-endometrial inter-relationship in the rat and guinea pig. *Anat. Record*. **104**: 331.
2. FAWCETT, D. W., G. B. WISLOCKI & C. M. WALDO. 1947. The development of mouse ova in the anterior chamber of the eye and in the abdominal cavity. *Am. J. Anat.* **81**: 413.
3. CHANG, M. C. 1950. Development and fate of transferred rabbit ova or blastocysts in relation to the ovulation time of recipients. *J. Exptl. Zool.* **114**: 197.
4. McLAREN, A. & D. MICHIE. 1956. Studies on the transfer of fertilized mouse eggs to uterine foster-mothers. *J. Exptl. Biol.* **33**: 394.
5. NOYES, R. W. & Z. DICKMANN. 1957. Proliferative changes in the trophoblast following ovariectomy and fetectomy in the rat. *Cancer Research*. **17**: 1054.
6. HERTIG, A. T. & H. W. EDMONDS. 1940. Genesis of hydatidiform mole. *A. M. A. Arch. Pathol.* **30**: 260.
7. HUGGETT, A. ST. G. & J. J. PRITCHARD. 1945. Experimental foetal death: the surviving placenta. *Proc. Roy. Soc. Med.* **38**: 261.
8. SELYE, H. J., J. B. COLLIP & D. L. THOMPSON. 1935. Endocrine inter-relationships during pregnancy. *Endocrinology*. **19**: 151.
9. FAWCETT, D. W. 1950. The development of mouse ova under the capsule of the kidney. *Anat. Record*. **108**: 71.
10. BRIDGMAN, J. 1948. A morphological study of the development of the placenta of the rat. *J. Morphol.* **83**: 61.
11. MCKAY, D. G., A. T. HERTIG, E. C. ADAMS & M. V. RICHARDSON. 1958. Histochemical observations on the human placenta. *Obstet. & Gynecol.* **12**: 1.

Discussion of the Paper

M. W. ORSINI (*Department of Anatomy, University of Wisconsin, Madison, Wis.*): Since we are discussing fundamental concepts concerning trophoblast, I propose to present some observations made on invasion and transport in the hamster.¹ The hamster has a gestation period of 15 days and 7 to

17 hours. During this period the trophoblastic cells invade the maternal vascular system in an orderly sequence and penetrate as far as the superior vessels of the mesometrium and even into the vessels near the ovary. They increase up to the time of parturition, decreasing thereafter, but are still present during the third week *post partum*.

This invasion of maternal blood channels is always via the arteries, never the veins. I have checked this carefully by study of serial sections of mater-

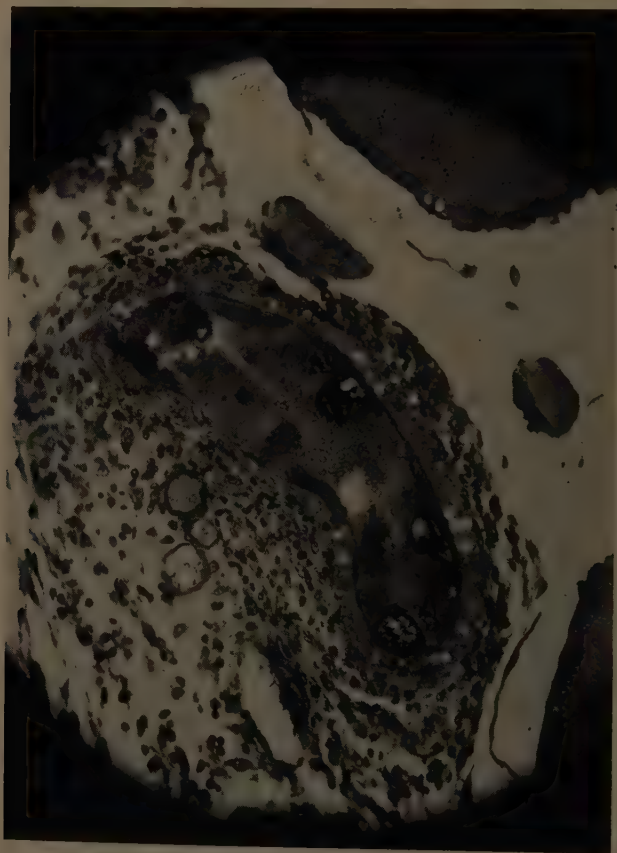


FIGURE 1. Reproduced by permission from *The American Journal of Anatomy*.¹

pregnant uteri with attached mesometria and have never seen any of these cells within veins. FIGURE 1 shows the mesometrial portion of a circumferential artery at 11 days and 12 hours; this is obviously an artery and the endothelium is distinct beneath the giant cells.

Invasion of arteries means, of course, invasion against the force of the current, suggesting a rheotropism. Migration into the arteries may possibly be effected by a stereotropic mechanism: all the invasive cells appear to be in contact at some point with the vessel wall. The fact that these cells are

not dislodged when the arterial system is injected with India ink via the aorta is additional evidence of this relationship.

Moreover, in late gestation and *post partum* stages the invaded arteries change, or are changed by this invasion. Many come to resemble veins, and it is only by the use of serial sections that the true nature of the vessel may be determined. FIGURE 2 shows a cross section of an artery in the superior mesometrium at 14 days and 10 hours. Adjacent sections of this same vessel

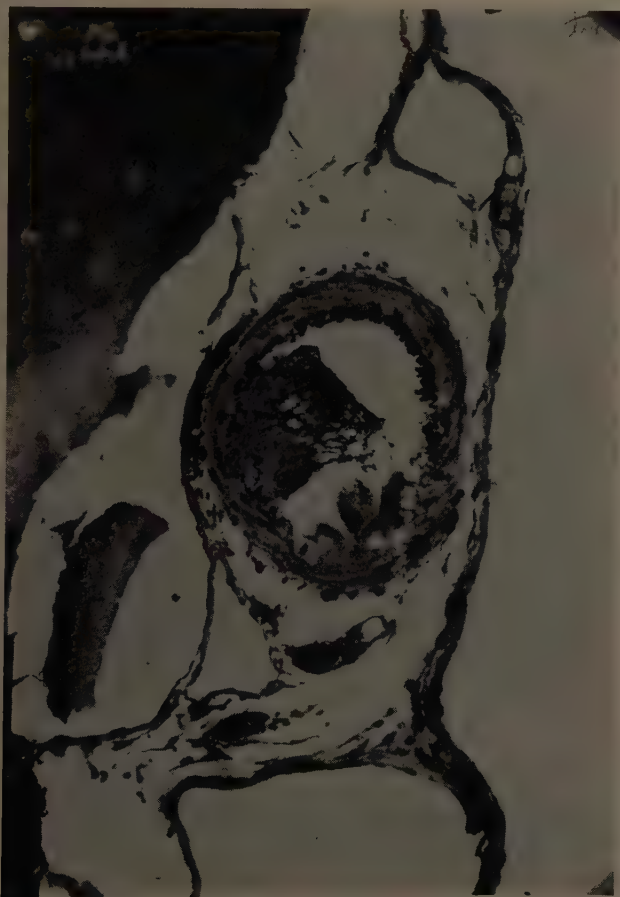


FIGURE 2. Reproduced by permission from *The American Journal of Anatomy*.¹

toward the interembryonic area show a perfectly normal artery, the entire wall being like the superior portion of the vessel in this figure. Here, in this region, are three giant cells; the arterial wall beneath these cells has lost its characteristic appearance and is thinner than that in the superior region. FIGURE 3 shows a portion of this same artery nearer the placenta proper. Here the vessel is dilated, has lost all resemblance to an artery, and closely resembles a vein. The invasive cells in this region are large flattened structures.

So, in summary, I stress the arterial nature of the invasion and the change within the arterial walls.

In conclusion I shall mention, apropos of the work on implantation and the excellent paper by B. G. Böving, the studies of delayed implantation being carried on at the University of Wisconsin by R. K. Meyer and his associates² and in Europe by a group of French scientists.³ In the rat, ovariectomized in early pregnancy and given daily doses of progesterone



FIGURE 3. Reproduced by permission from *The American Journal of Anatomy*.¹

the blastocyst does not implant, but may remain viable in the uterus for at least as long as the entire normal period of gestation. It implants when estrone is added to the progesterone. This study is being extended to other forms. I draw your attention to this simply as another possible research tool or technique that may aid in elucidating the mechanism of nidation.

References

1. ORSINI, M. C. 1954. The trophoblastic giant cells and endovascular cells associated with pregnancy in the hamster, *Cricetus auratus*. *Am. J. Anat.* **94**: 273-331.
2. COCHRANE, R. L. & R. K. MEYER. 1957. Delayed nidation in the rat induced by progesterone. *Proc. Soc. Exptl. Biol. Med.* **96**: 155-159.
- 2a. MEYER, R. K., M. R. N. PRASAD & R. L. COCHRANE. 1958. Delayed implantation in the rat induced by autographs of the hypophysis. *Anat. Record*. **130**: 339.
3. CANEVINC, R., M. LAFFARGUE & G. MAYER. 1956. Nidations retardées chez la rat castrée et injectée de progestérone. Influence du moment de la castration sur la chronologie de l'ovoimplantation. *Compt. rend. soc. biol.* **150**: 2208-2212.

HORMONAL ACTIONS OF CHORIONIC GONADOTROPIN*

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Many students of the evolution of viviparity and of the endocrinology of reproduction have become impressed with what I term Amoroso's basic law of the comparative physiology of reproduction: "With the transition from oviparity to viviparity in mammals and also in fishes and reptiles, there have occurred profound changes in the nature of the egg and in the structure and physiology of the female animal, particularly of the reproductive tract. It would be difficult to imagine that these complex and highly integrated changes and events, observed in vertebrates, particularly in reptiles, could have come about otherwise than through many separate lines of evolutionary specialization. Nevertheless, it is clear that none of these evolutionary innovations could have occurred in the complete absence of various interactions between tissues and hormones."²

During the past ten years I have pursued the several metabolic alterations of the reproductive tract as they are influenced by the hormones and/or their metabolites acting in concert.³⁹ Specifically, I have become interested in the interactions between tissues and hormones. Although we have all learned more of the physiological actions of the estrogens, progestogens, and androgens, much less is known of the adeno-hypophyseal and placental gonadotropins. The present report is concerned directly with a series of related experiments that have been devised with the purpose of elucidating the physiological role of a placental gonadotropin of the human, chorionic gonadotropin (HCG) on the reproductive system. Giving added impetus to this study are the numerous lamentable lacunae in our understanding of the real purpose of this placental gonadotropin in the body fluids of the pregnant human female, as well as its role during several of the known pathological variants.

MATERIALS AND METHODS

The immature albino rats‡ used in these experiments were derived from the Sprague-Dawley strain. The adult albino rats§ used for the pseudo-pregnancy studies were also descendants of the Sprague-Dawley strain.

STUDIES ON IMMATURE RATS

The immature animals were selected at random, earmarked, placed 6 to a $6 \times 9 \times 19$ -inch wire-mesh cage, and were fed the standard Purina Lab Chow

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† These studies carried out under the tenure of a Lederle Medical Faculty Award, made by the Lederle Laboratories Division of American Cyanamid Company, Pearl River, N. Y.

‡ Obtained from Charles River Breeding Laboratories, Brookline, Mass.

§ Obtained from Harvard Colony, Cambridge, Mass.

and tap water *ad libitum*. All hypophysectomies were performed by the parapharyngeal approach 1 week prior to placing them on these experiments. These animals were similarly maintained, except for the addition of 1 per cent glucose in the tap water.

For clarity and ready reference, the injection schedules are given in each table. The injections were administered subcutaneously and, when more than one substance was administered, different injection sites were used. All necropsies were performed 12 hours after the last injection; the endocrine organs were removed quickly, weighed to the nearest 0.2 mg. on a Roller-Smith torsion balance, and prepared for histological examination.

Both the HCG and the 4-aminopteroylglutamic acid (4-amino-PGA, or Aminopterin) were dissolved separately in physiological saline solution.

STUDIES IN MATURE RATS

Adult albino rats 100 days of age and weighing 200 to 225 gm. were used for this phase of the study. The animals were selected at random, ear-marked, placed 6 to a 6 × 9 × 18-inch wire-mesh cage, and were fed the standard Purina Lab Chow and tap water *ad libitum*. Vaginal smears were taken at least twice daily to ascertain the regularity of their estrous cycles. The aqueous solutions and HCG hormone were administered subcutaneously in a single injection in 0.1 cc. vehicle per injection.

Pseudopregnancy was induced in estrous albino rats approximately 100 days of age by electric stimulation of the uterine cervix. Four days later the animals so stimulated were given single, subcutaneous injections of HCG (Antuitrin-S.*) of a dosage range from 1 to 100 international units (I.U.). The duration of pseudopregnancy was measured by examining vaginal smears of the experimental animals twice daily until a proestrous or estrous smear was found.

In addition to the vaginal smear studies, it was of particular interest to ascertain the effects of the chorionic gonadotropin on the ovaries. Three to 5 rats were selected at random from each dosage level and were sacrificed either at 18 or 24 hours after the injection. Serial sections of the ovaries

* Product of Parke, Davis & Company, Detroit, Mich.

† The League of Nations Health Organization (M. 40, April 1939) announced the establishment of a standard International Unit for HCG: "The specific gonadotrophic activity of 0.1 mg. (100 gamma) of the standard preparation shall be the International Unit for recording the activities of all gonadotrophic preparations of human urine of pregnancy, but only of such."

Recommendations for the use of the standard in biological assays taken from the *Report of the Conference, Bulletin of the Health Organization of the League of Nations*, 7, 889, 1938 are as follows: "The only test for the comparative determination of gonadotrophic activity, in units as above defined, on which the conference has evidence to justify recommendation, depends on:

"(a) The observation of a direct or indirect effect, shown by morphologic changes in the gonads.

"(b) The observation of secondary changes, in the accessory reproductive organs, in animals not deprived of their gonads. When this type of test is used, the absence of substances directly causing such changes in the accessory reproductive organs should be assured by control tests of animals deprived of their gonads."

and attached oviducts were stained with hematoxylin and eosin and examined for evidences of ovulation and luteinization.

RESULTS

Experimental Results in Immature Female Rats

Influence of small doses of HCG. Previously, results obtained in Hisaw's laboratory had indicated that small doses (<5.0 I.U.) of HCG elicited a marked effect on the uterine weight of intact, immature rats. Therefore, it appeared to be of interest to ascertain the effects of small doses of this placental hormone on the different aspects of the sex tract and adrenal glands of intact, immature albino rats. These results indicate that 4 daily doses of 0.10 I.U. of HCG, when given in 2 equally subdivided injection amounts at 9:30 A.M. and 4:30 P.M., induce a slight but positive increase on both the ovarian and oviductal weights ($p = <0.05$). The uterine weight in this regimen of HCG was greatly increased (113 per cent and 138 per cent) when compared respectively with actual weights of the ovaries and/or those of the paired ovaries: body weights (milligrams per cent) of the aqueous-treated controls (TABLE 1). The adrenal glands also increased 42 to 55 per cent over those of the nonhormonally-treated controls (TABLE 1). The histological preparations reveal that both the ovaries and the oviducts show stimulation by HCG. The ovaries of the hormonally treated animals contained many vesicular follicles, and the oviducts gave indication of estrogenic stimulation. Both the uteri and adrenal glands present evidence of marked estrogenic stimulation: the endometrial luminal epithelium appearing quite columnar, and the adrenal cortex containing a thickened zona fasciculata.

Daily doses of 0.50 and 0.75 I.U. of HCG produced ovaries that showed an increase of 50 to 77 per cent and 35 to 78 per cent, respectively, over those of the aqueous-treated controls (TABLE 1). The oviducts, however, were well within the range of the 0.10 I.U. of HCG-treated animals. The uteri of rats given the 0.50-I.U. daily dose were only slightly heavier than those on the 0.10-I.U. dosage, whereas the response of the uteri of the animals given 0.75 I.U. was almost identical with that produced on the 0.10-I.U. treatment (TABLE 1). The adrenal glands on the 0.50-I.U. and 0.75-I.U. treatments showed stimulation histologically, but the gravimetric data were less indicative of stimulation.

Rats given 1.0 I.U. of HCG subcutaneously daily had larger uteri and adrenal glands than those given smaller amounts. This is likewise true of the actual weights, except for the uterine weights (TABLE 1). Histologically, all of the components of the female reproductive tract showed evidence of hormonal stimulation: the ovaries showed evidence of preovulatory swelling and/or ovulation; the oviducts, uteri, and vaginae presented evidence of estrogenic stimulation. The adrenal glands were quite comparable histologically to those on the 0.10-I.U. dosage.

Influence of large doses of HCG. Having established the minimal effective dosage of HCG that increases the organ weights and elicits definite stimulation by histological examination, it then appeared of interest to ascertain

the effects of large amounts (2.5 to 20.0 I.U.) on the sex tract and adrenal glands of intact, immature female rats. These data reveal that the response to 2.5 I.U. is quite comparable to that obtained with 1.0 I.U. (TABLES 1 and 2). The response to 5.0 I.U. indicates an increased effect of the administered hormone on the ovaries and uteri, but not on the oviducts nor the adrenal glands. Surprisingly, the oviducts were intermediate in response between the aqueous-treated and those of the 1.0-I.U. HCG groups. This was likewise true of the adrenal glands (TABLE 2). A further increase in

TABLE 1
ACTION OF SMALL AMOUNTS OF HUMAN CHORIONIC GONADOTROPIC HORMONE (HCG)
ON THE SEX TRACT AND ADRENAL GLANDS OF INTACT, IMMATURE FEMALE RATS*

Treatment HCG in I.U. daily for 4 days	No. Rats	Organ weights (averages)							
		Ovary		Oviduct		Uterus		Adrenals	
		mg.	mg. %†	mg.	mg. %	mg.	mg. %	mg.	mg. %
Pretreatment controls									
Necropsies (N) performed on 25th day of age (D.A.).....	10	20.2†	33.4	8.6	14.09	34.6	56.7	17.1	28.1
Posttreatment (aqueous vehicle) controls									
R = 0.1 cc. aqueous vehicle A.M. and P.M. 25 to 28; N 29 D.A....	11	18.6	22.7	9.7	11.1	44.0	50.5	17.3	19.3
0.1 I.U.....	10	23.7	30.0	13.9	17.5	94.3	119.3	24.6	31.1
0.5 I.U.....	11	27.3	31.0	14.9	16.9	116.7	132.6	22.7	25.1
0.75 I.U.....	11	32.0	40.5	12.7	16.1	94.9	120.1	18.9	23.1
1.0 I.U.....	15	40.7	50.1	20.0	24.9	109.8	134.9	25.0	30.1

* Injections administered on twenty-fifth to twenty-eighth D.A.; N performed 12 hours after last injection, day 29.

$$\dagger \text{ Mg. \%} = \frac{\text{organ wt.}}{\text{body wt.}} \times 100$$

‡ Arithmetic average; all standard errors were less than 10 per cent arithmetic average

ovarian and uterine weight was obtained with a daily treatment of 10.0 I.U. of HCG. The oviducts were quite comparable to those in the 5.0-I.U. group, but the adrenal glands resembled those of the 1.0-I.U.-treated animals. A plateau effect seems to be achieved with daily doses > 10.0 I.U. and for simplicity and conciseness, only the results of the 10.0- and 20.0-I.U. daily-treated animals are presented in TABLE 2. Examination of these data reveals that 10.0 to 20.0 I.U. of HCG given daily for 4 days results in a 26. to 280 per cent increase in ovarian weight, 65 to 72 per cent increase in oviductal weight, 180 to 187 per cent increase in uterine weight, and a 16 to 20 per cent increase in adrenal weight (TABLE 2).

The histological effects were equally interesting. Of greater significance is the fact that marked ovarian follicular stimulation was obtained with 0.1 I.U. of HCG daily. Ovulation and luteinization were obtained in more than 50 per cent of the animals on the 2.5-I.U. regimen and in almost 100 per cent of the rats given 5.0 to 20.0 I.U. of HCG. Vaginal cornification was produced in 70 per cent of the animals given 0.10 to 1.0 I.U. daily and in 100 per cent of the rats given doses > 1.0 I.U. of HCG daily for the 4-day injection period. The adrenal glands consistently presented evidence of

TABLE 2
INFLUENCE OF HUMAN CHORIONIC GONADOTROPIC HORMONE (HCG) ON THE SEX TRACT AND ADRENAL GLANDS OF INTACT, IMMATURE FEMALE RATS*

Treatment HCG in I.U. daily for 4 days	No. Rats	Organ weights (averages)							
		Ovary		Oviduct		Uterus		Adrenals	
		mg.	mg. %†	mg.	mg. %	mg.	mg. %	mg.	mg. %
Retreatment controls									
Necropsies (N) performed on 25th day of age (D.A.).....	17	22.0†	34.0	10.4	16.0	44.0	52.0	21.0	33.0
Posttreatment (aqueous-treated) controls									
R ₁ = 0.1 cc. aqueous vehicle A.M. and P.M. 25 to 28; N 29 D.A.....	16	21.0	25.5	9.7	11.8	55.7	68.8	20.6	25.5
1.0 I.U.....	15	40.7	50.1	20.0	24.9	109.8	134.9	25.0	30.7
2.5 I.U.....	15	48.6	57.6	21.8	25.8	128.8	153.9	24.5	29.1
5.0 I.U.....	15	53.3	64.6	15.8	19.3	145.6	179.9	22.1	26.8
10.0 I.U.....	13	74.5	97.4	14.5	19.0	159.8	208.3	25.1	32.8
20.0 I.U.....	16	76.3	97.2	16.0	20.3	154.6	196.9	24.0	30.6

* Injections administered on twenty-fifth to twenty-eighth D.A.; N performed 12 hours after last injection, day 29.

† Mg. % = $\frac{\text{organ wt.}}{\text{body wt.}} \times 100$

‡ Arithmetic average; all standard errors were less than 10 per cent arithmetic average.

estrogenic stimulation, the estrogen being released by the synergism of the HCG with the animal's follicle-stimulating hormone (FSH).

Influence of HCG on the sex tract and adrenal glands of hypophysectomized, immature female rats. In an effort to test certain features of the *modus operandi* of HCG it seemed most desirable to use hypophysectomized, immature rats. In so doing, an opportunity would be presented to ascertain the more precise nature of this hormone, as well as an indication of the possible intervention of the adeno-hypophyseal gonadotropins in the intact animal. It will be recalled that the greatest effects achieved in intact animals were

with 4 daily doses of 10.0 I.U. of HCG (TABLE 2). Therefore, it seemed appropriate to employ a dosage range of 2.0 to 10.0 I.U. daily. These data reveal that HCG displays the ability to stimulate ovarian weight and to induce partial repair of the hypophysectomy-induced ovarian atrophy. The ovarian weight of the 10.0-I.U.-treated group was increased 85 to 107 per cent over that of aqueous-treated animals (TABLE 3). There was no significant change in the weights of the oviducts, and only a very slight but statistically significant increase in uterine and adrenal weights was induced with the 10.0-I.U. dosage (TABLE 3).

TABLE 3

INFLUENCE OF HUMAN CHORIONIC GONADOTROPIC HORMONE (HCG) ON THE SEX TRACT AND ADRENAL GLANDS OF HYPOPHYSECTOMIZED, IMMATURE FEMALE RATS*

Treatment HCG in I.U. daily for 4 days	No. Rats	Organ weights (averages)							
		Ovary		Oviduct		Uterus		Adrenals	
		mg. (Actual)	mg. %†	mg. (Actual)	mg. %	mg. (Actual)	mg. %	mg. (Actual)	mg. %
Pretreatment controls Necropsies (N) per- formed 36th day of age (D.A.).....	5	10.0‡	13.3	3.8	5.1	18.7	24.9	10.2	13.6
Posttreatment (aqueous-treated) controls R = 0.2 cc. aqueous vehicle A.M. and P.M. 36 to 39; N 40 D.A.....	6	12.3	15.4	6.4	8.0	19.7	24.6	13.0	16.5
2 I.U. daily.....	6	13.8	19.4	6.9	9.7	14.2	20.0	11.9	16.8
4 I.U. daily.....	6	16.6	22.4	6.1	8.2	15.6	21.1	12.6	17.0
10 I.U. daily.....	6	22.7	31.97	6.3	8.9	22.3	31.4	15.0	21.1

* Animals hypophysectomized on twenty-fifth D.A.; all animals started on HCG regimen on thirty-sixth day; injected twice daily subcutaneously for 4 days; N performed 12 hours after last injection, on fortieth D.A.

$$\dagger \text{Mg. \%} = \frac{\text{organ wt.}}{\text{body wt.}} \times 100$$

‡ Arithmetic average; all standard errors were less than 10 per cent arithmetic average and are therefore not included.

The histological sections revealed repair of the deficient (atrophied) ovarian interstitial tissue and a conspicuous lack of ovarian follicular development. Especially noteworthy were: (1) the very minimal ovarian hypertrophy, (2) the slight but positive uterine stimulation, (3) the lack of oviduct stimulation and (4) the slightly stimulated adrenal cortex, particularly the zona fascicu-

ata. The weights of the ovaries are somewhat misleading, especially since they show a 100 per cent increase over the aqueous-treated, hypophysectomized female rats, whereas the histology indicates only slight stimulation. The ovaries from the animals given four daily doses of 10.0 I.U. of HCG contained no more than 1 or 2 vesicular follicles in sharp contrast to the numerous and healthy vesicular follicles and corpora lutea in the HCG-treated, intact animals. These effects are in marked contrast to those observed in the intact animal.

Influence of 4-amino-PGA (Aminopterin) on HCG-induced changes in immature female rats. Previously Velardo and Hisaw had reported that 4-amino-PGA possesses the ability to interfere with estrogen in uterine growth³⁵ and

TABLE 4
INFLUENCE OF AMINOPTERIN ON HUMAN CHORIONIC GONADOTROPIC
HORMONE-INDUCED CHANGES IN IMMATURE FEMALE RATS*

Treatment daily for 4 days	No. Rats	Averages											
		Ovary		Oviduct		Uterus		Adrenals		Pituitary		Thyroid	
		mg.	mg. %†	mg.	mg. %	mg.	mg. %	mg.	mg. %	mg.	mg. %	mg.	mg. %
Posttreatment (aqueous treated) controls R = 0.2 cc. aqueous vehicle A.M. and P.M., 25 to 28; N 29 D.A.....	17	22.0†	34.0	10.4	16.0	44.0	52.0	21.0	33.0	3.0	3.7	5.2	6.8
1 I.U. HCG daily.....	15	40.7	50.1	20.0	24.9	109.8	134.9	25.0	30.7	3.2	4.3	6.6	8.9
5 I.U. HCG.....	15	53.3	64.6	15.8	19.3	145.6	179.9	22.1	26.8	3.4	4.6	7.2	9.0
0.01 mg. Aminopterin.....	8	18.1	24.8	8.7	11.9	31.1	42.6	21.3	29.2	3.0	4.1	5.4	7.4
0.02 mg. Aminopterin.....	8	18.6	24.8	7.3	9.7	23.8	31.7	16.6	22.1	3.2	4.3	5.9	7.9
0.01 mg. Aminopterin plus 1 I.U. of HCG.....	8	31.6	42.1	14.2	19.0	76.6	102.1	19.5	26.0	4.5	6.0	8.4	11.2
0.01 mg. Aminopterin plus 5 I.U. of HCG.....	8	49.7	64.5	17.7	23.0	130.2	169.1	22.3	29.0	5.0	6.5	8.3	10.8

* Injections administered on twenty-fifth to twenty-eighth D.A.; N performed 12 hours after last injection, day 29.

† Mg. % = $\frac{\text{organ wt.}}{\text{body wt.}} \times 100$

‡ Arithmetic average; all standard errors were less than 10 per cent arithmetic average.

progesterone in the Hooker-Forbes response in Swiss albino mice³⁷ and in decidual tissue development in rats.³⁶ Thus, it naturally followed that: (1) since HCG is known to cause the secretion of estrogen and progesterone by the rodent and primate corpus luteum, and (2) since 4-amino-PGA has been used in cancer chemotherapy, it might prove of value to ascertain the effect of 4-amino-PGA on the changes elicited by HCG.

Four daily doses of either 0.01 or 0.02 mg. 4-amino-PGA were quite effective in depressing the normal growth and development of the sex tract and the adrenal glands of intact, immature albino rats (TABLE 4). Neither the pituitary nor the thyroid glands were affected by the 4-amino-PGA. Combinations of 0.01 mg. of 4-amino-PGA and 1.0 I.U. of HCG effected changes in the sex tract and adrenal glands that were approximately 33 per cent less

than those produced on the 1.0 I.U. of HCG regimen alone (TABLE 4). Conversely, the combined effects elicited weight changes of pituitary glands and thyroids that were almost consistently 33 per cent heavier than those on the 1.0 I.U. of HCG treatment (TABLE 4). That the response on the sex tract and adrenal glands is one of competitive inhibition is borne out in the results obtained from the group given 0.01 mg. of 4-amino-PGA and 5.0 I.U. of HCG concurrently. These data reveal that the inhibition previously observed is either nonexistent or only very slight, but not statistically distinguishable from those given 5.0 I.U. of HCG daily for 4 days without 4-amino-PGA (TABLE 4). Of singular significance is the increased weight response of both the pituitary and thyroid glands, an effect observed in the previous group on the 0.01 mg. of 4-amino-PGA and the 1.0 I.U. of HCG treatment (TABLE 4).

Experimental Results in Adult Albino Rats

Influence of HCG on the length of pseudopregnancy. Burdick and Crump reported that a single injection of HCG-induced ovulation in pregnant mice, and that this ovulation did not interfere with the pregnancy.⁸ It thus became of interest to ascertain the effects of HCG on the length of pseudopregnancy in adult albino rats.

TABLE 5
INFLUENCE OF CHORIONIC GONADOTROPIN ON THE LENGTH OF PSEUDOPREGNANCY
IN THE ADULT ALBINO RAT

Number of rats	Dosage in I.U.	Mean length of pseudopregnancy in days	Standard error in days	Range in days
65	0.0	13.0	0.04	12-14
8	1.0	12.0	0.4	11-14
7	2.5	13.0	0.5	11-15
7	5.0	14.0	0.4	13-16
8	10.0	13.6	0.5	11-16
10	15.0	17.9	0.6	14-20
11	20.0	17.5	0.8	12-21
11	25.0	16.6	0.9	12-22
10	30.0	18.4	0.7	14-21
10	35.0	18.3	1.0	12-23
10	40.0	18.2	0.9	13-23
11	45.0	18.2	0.8	14-23
9	50.0	19.2	0.7	15-22
8	60.0	19.5	0.5	18-22
8	80.0	19.3	0.6	17-23
8	100.0	20.3	0.7	17-23

Estrous albino rats approximately 100 days of age were made pseudopregnant by electrically stimulating the uterine cervixes and, 4 full days later, they were given a single subcutaneous injection of HCG in a dosage range of 1.0 to 100.0 I.U.

These results indicate that ovulation was induced in pseudopregnant rats receiving 15.0 to 100.0 I.U. of HCG. The normal mean length of pseudopregnancy (13.0 days) was prolonged to 17.3 days by 15.0 to 25.0 I.U. of HCG. Injections of 30.0 to 50.0 I.U. increased the length of pseudopregnancy to approximately 18.7 days, and doses of 60 to 100 I.U. resulted in a period of pseudopregnancy lasting 19.3 to 20.3 days (TABLE 5).

Experimental Results on HCG Obtained by Others

Effects on ovarian growth and ovulation. Burdick and Crump⁸ and Enders *et al.*¹⁵ demonstrated that HCG in very minute doses (1.5 to 15.0 I.U.) readily induces ovulation during different stages of pregnancy in the mouse and during pseudopregnancy in the rat. In both instances there were no deleterious effects on the pregnancy or the pseudopregnancy.

Newton,²⁶ in an extensive survey of research on hormones and the placenta down to 1938, reviewed the early work on HCG. Suffice it to say that HCG displays the property of increasing the weight of the ovary and inducing preovulatory changes.²⁶ Fluhman reported that an augmentative effect exists when HCG and estrogen are injected concurrently in immature rats.¹⁶ Albert and Money evaluated the comparative effect of HCG and equine gonadotropin on the rate of ovarian growth; they concluded that HCG is quite active as an ovarian growth promoter in 21-day-old rats.¹ More recently, Steelman and Pohley have proposed an assay test for FSH based on the augmentative effects of HCG.³²

Effects on menstruation. Hisaw devised a large number of experiments to test the action of HCG on the functional life of the corpora lutea of monkeys (*Macaca mulatta*).²¹ Adult animals whose menstrual cycles had been observed for two or more years were used in most of the experiments. Some preliminary observations were made on a few monkeys whose menstrual histories were not studied. In all instances, however, the presence of a corpus luteum was determined by laparotomy during the luteal (progravid) phase of the cycles.

The first day on which external bleeding (from menstrual source) was noticed was used as the first day of the menstrual cycle, and all succeeding events and dates during the normal and experimentally modified cycles were counted from that time. Those monkeys in which obviously functional corpora lutea were observed and/or determined were started on daily subcutaneous injections of 300.0 to 1000.0 I.U. of HCG and continued until the onset of the next menstrual period.

Fifteen monkeys whose menstrual cycles (47 cycles) averaged 26.5 days were given daily subcutaneous injections of HCG beginning between the eighteenth and twenty-fourth days and continued until the next menstruation. The average length of the experimental cycles was extended to 41.9 days. The lengthening of the cycles was associated with a continuation of function of the luteal tissue, as evidenced by the progestational endometria and other luteal effects. The morphologic appearance of the corpora lutea was modified to that typical of pregnancy.

In another series of experiments, in which corpora lutea were produced in

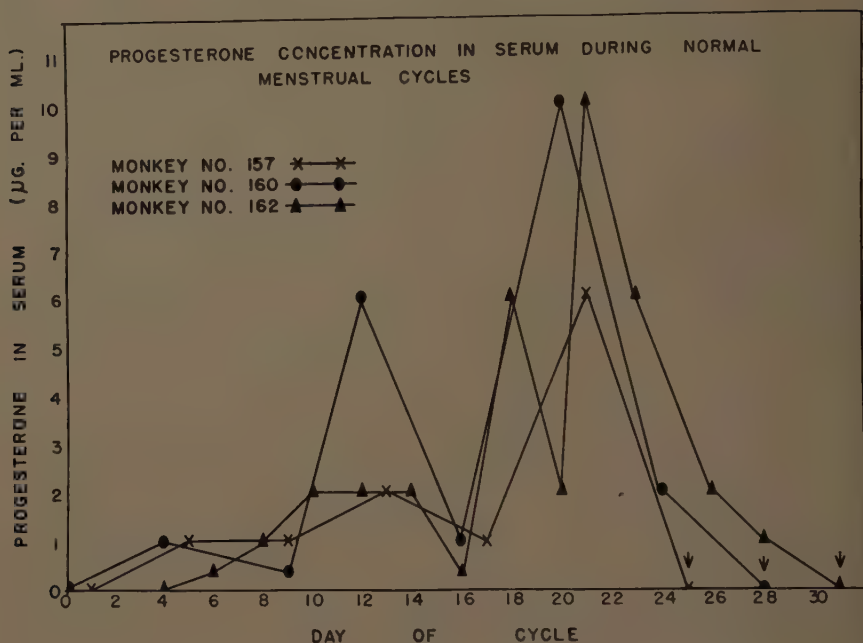


FIGURE 1. From Bryans;⁷ reproduced by permission from *Endocrinology*.

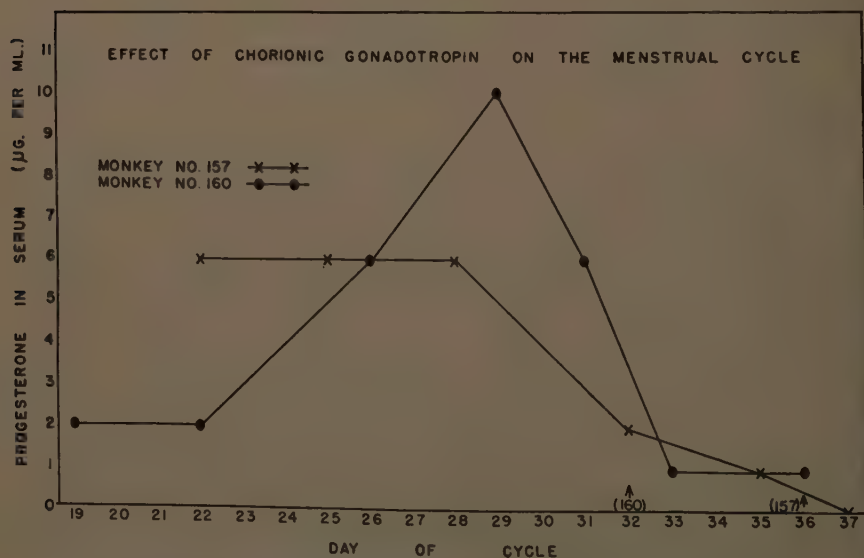


FIGURE 2. From Bryans;⁷ reproduced by permission from *Endocrinology*.

4 monkeys by giving pituitary gonadotropic hormones and blood serum of pregnant mares, Hisaw observed that HCG likewise extended the functional life of luteal tissue and delayed the onset of menstruation in these 4 monkeys.²¹

It is of particular significance to point out that, while HCG prolonged the life of the functional corpus luteum and effected an elapsed period of time between the luteal life and the normally expected period of menstruation, adenyhypophyseal luteotropin (LtH, or prolactin) failed to do so.²¹

Effects of HCG on ovarian hormonal secretions. In a follow-up of Hisaw's observations that HCG prolongs the morphologic and physiological aspects

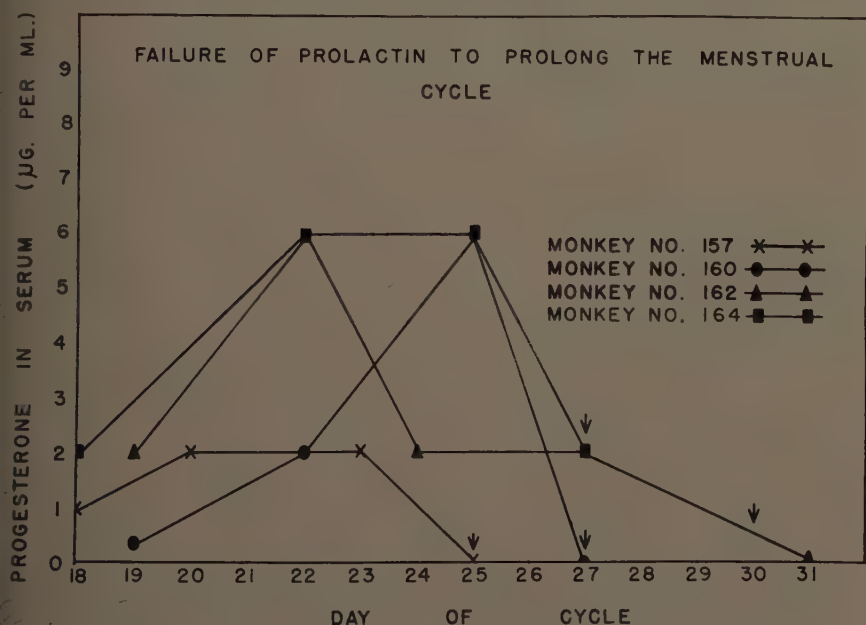


FIGURE 3. From Bryans;⁷ reproduced by permission from *Endocrinology*.

of the corpus luteum in monkeys, Bryans determined that Hisaw's report lends itself to complete duplicability, and adduced further experimental evidence showing that the prolongation of luteal life was associated with the continued presence of progesterone in the blood, as measured by the Hooker-Forbes technique (FIGURES 1 and 2).⁷ The secretory life of the corpus luteum was *not* prolonged in four monkeys given prolactin during the luteal phase of the menstrual cycle. Menstruation occurred at the normal time and was preceded by the normal decrease of progesterone in the blood (FIGURE 3).

In Hisaw's experiments it was observed that the reproductive tract and sexual skin of monkeys whose menstrual cycles have been prolonged by HCG manifested conditions comparable to those induced in ovariectomized animals given simultaneous injections of estradiol-17 β and progesterone. Hisaw concluded that the corpus luteum may be the chief source of both estro-

gen and progesterone from about the fourteenth to the thirtieth day of pregnancy.²¹

Effects of HCG during pregnancy. The first detectable effect of HCG in the pregnant human patient is one of a diagnostic nature. Aschheim and Zondek first demonstrated that in pregnancy, and only two weeks after the first missed menstrual period, there was a large quantity of gonadotropin material in the blood and urine.³ Their initial observations led to the use of the so-called A-Z test as a diagnostic test of pregnancy. Since their original discovery, numerous modifications and different assay animals have been proposed as refinements of their methods.^{10, 12, 14, 22, 24, 29}

In a number of experiments concerned with the maintenance of the corpus luteum and the physiological actions of progesterone, Bradbury and his associates⁶ determined that HCG will prolong the functional activity of the corpus luteum of the human and thereby give rise to a condition of pseudo-pregnancy, an effect similarly observed in the laboratory rat, but undoubtedly via different mechanisms. These same workers also reported that HCG failed to influence the developing follicle and likewise failed to revive a regressing corpus luteum or to affect the secretory (progravid) endometrium in an ovariectomized subject. Thus, they concluded that, since the action of HCG was limited to a pre-existing, functional corpus luteum, the luteotropic agent resident in this placental hormone is responsible for the early development of the corpus luteum of pregnancy.

Rosenfeld and his collaborators have unequivocally shown that HCG, when administered to pregnant rats, induces inhibition of parturition and causes early death of fetuses and their resorption. These same workers also reported a marked stimulation of the ovaries with formation of additional corpora lutea, follicular cysts, and occasional hemorrhagic follicles.²⁸ Burrows⁹ and, more recently, Velardo³⁹ have summarized the effects of HCG on the reproductive tracts of female animals.

Effects of HCG in males. Noble and Plunkett have recently summarized the actions of HCG in male laboratory animals.²⁷ In hypophysectomized male rats HCG repairs and stimulates the interstitial cells of Leydig and allows for androgen secretion to occur therefrom. In the intact animal all testicular elements undergo stimulation, thereby causing testicular hypertrophy and increased stimulation of the secondary sex organs (seminal vesicles and the prostate glands). It is now firmly established that the synergism observed in the intact male rat is due to the intervention of the adeno-hypophyseal gonadotropins.

Harrison has recently reported on the effect of prolonged injections of HCG on the rat testis.¹⁸ In his report before the British Fertility Association (Regents Park, London, England, June 1956), Harrison reviewed the previous reports which suggested that injections of HCG for longer than 2 to 3 weeks have no action on, or produced regressive changes in, the rat testis. Utilizing rats from a colony known to have a normal testicular histology, Harrison elected to conduct his experiments thus: daily subcutaneous injections of 80.0 I.U. (Pregnyl*) were administered to rats for

* Product of Organon Laboratories, Ltd., London Division, London, England.

3 calendar months; their littermates were given sterile pyrogen-free distilled water. His results showed that there was no significant difference in organ weights in the 2 groups of rats. The testes of all animals showed minor pathological changes: phytoid vacuolation of spermatogonia and primary spermatocytes with hypervesicular nuclei and an increased amount of interstitial fluid at the periphery of the testis. These changes were identical in both groups of rats. Harrison concluded that these changes probably were produced by the stress of repeated daily injections.

Maddock and Nelson²⁵ obtained evidence that shows that urinary estrogen is increased from 5 to 16 times pretreatment levels in each of 5 men given 5000 units of HCG (APL*) 3 times weekly for 47 to 65 days. Urinary 17-ketosteroid excretion increased to approximately twice that of pretreatment levels, but these levels were variable and were in no way related to the changes in estrogen secretion. Repeat testicular biopsy specimens were obtained from 3 of the 5 patients at the conclusion of the treatment. Leydig cells in all 3 men were increased greatly in numbers and displayed histochemical changes indicative of enhanced secretory activity. The seminiferous tubules were decreased in size and revealed basement membrane hyalinization, peritubular fibrosis, cessation of active spermatogenesis, and necrosis and sloughing of germinal cells. The number of sperm in the seminal fluid decreased to almost zero. There were no obvious changes in numbers or histochemical observations in the Sertoli cells of these men. Therefore, Maddock and Nelson concluded that: (1) the Leydig cells, rather than Sertoli or germinal cells, are the source of the estrogen elaborated by the human testis; (2) adult, functioning Leydig cells respond to HCG by secreting increased amounts of androgen and estrogen; (3) estrogen excretion is a more sensitive and reliable indicator of the response of Leydig cells to HCG than is 17-ketosteroid excretion, and (4) the seminiferous tubule damage, together with the concomitant decrease in number of sperm in seminal fluid that occurs during HCG administration is most probably due to suppression of adenohipophyseal FSH secretion by the increased levels of endogenous estrogen and androgen.²⁵

Finally, it should be stated that HCG has been used with a high degree of success in cryptorchidism in young boys 11 to 17 years of age.³¹ Usually 500 I.U. of HCG given intramuscularly twice a week for 2 months will induce descent of the testes. Some clinicians state that a hot bath may produce the same effect in young boys manifesting a mechanical obstruction in the area of the inguinal ring.

HCG in pathological variants. Numerous investigators have reported that abnormally high HCG titers are associated with clinically documented cases of hydatidiform moles^{4, 24} and of chorionepithelioma of the uterus and testis. High serum levels of HCG have also been reported in cases of pre-eclamptic toxemia and in diabetic pregnancy, but in such cases very little can be correlated with the HCG levels and the clinical course of the disease. It has been concluded that HCG estimations in cases of pre-eclamptic toxemias and diabetic pregnancies are of little or no value from the prognostic point of view.²⁴

* Product of Ayerst, McKenna and Harrison, Ltd., Toronto, Canada.

DISCUSSION

The experimental results and observations of the effects of HCG give added credence to the concept that the placenta functions as an endocrine organ during pregnancy. Halban's observations made in 1905 pointed up the endocrine relationships of the human placenta and provided, at the dawn of endocrinology, a concrete example of the fetal-maternal relationships and the importance of the placenta in this regard.¹⁷ Although the occurrence of gonadotropic activity in human pregnancy urine was first described by Aschheim and Zondek³ in 1927 to 1928, the source of this hormone was conjectural. Conclusive evidence now exists to show that the cytotrophoblastic tissue of the placenta is the tissue source of HCG.³³ Since these reports of classic value have appeared, HCG has been biologically characterized, assayed, and standardized in terms of international units.^{5, 10, 12, 14, 24, 27, 29} Admittedly, most of our information concerning this placental hormone has been derived from experiments in laboratory animals, but of greater significance is the impetus given to us for the clinical studies.

The results reported in this paper emphasize several points: (1) adeno-hypophyseal gonadotropins synergize with HCG and stimulate ovarian growth and induce ovulation; (2) HCG increases adrenal weight and the width of the zona fasciculata; (3) HCG stimulates the interstitial tissue of the ovaries in hypophysectomized rats, and (4) HCG induces ovulation and prolonged luteal function in pseudopregnant rats. The fact that HCG increases adrenal weight (function?) in intact rats that display a positive ovarian response (ovarian hyperemia and/or ovulation) gives added credence to the concept that the several modifications of the reproductive tract are governed by the sum total of all the hormones and/or their metabolites acting in concert.³⁹ Behrman and Niemann⁵ recently concluded that the adrenal cortex must be functioning and that it must produce a certain amount of cortisone for HCG to induce its effect on the ovary, thus providing confirmation of the previously described thesis.

One of the most significant and noteworthy observations made in the present study concerns the action of HCG on ovarian development, ovulation, and luteal function. Surprisingly enough, until the year 1944, there were no clear-cut demonstrable effects of HCG on the mother or fetus; consequently, Levin stated: "Therefore, until we have more evidence, it may be just as well to consider the appearance of chorionic gonadotrophin as a gift of nature designed to make easier the obstetrician's task in the diagnosis of early pregnancy. This much we know is true."²³ However, consider the recent observations of Hisaw,²¹ Hartman,^{19, 20} van Wagenen^{13, 34} and others. Our factual knowledge of HCG and placental function has increased tremendously since Levin's 1944 report.^{9, 39}

That decidual tissue has a real effect on the corpora lutea of pseudopregnant rats is amply demonstrated by the following observations: (1) massive decidual tissue prolongs the status of pseudopregnancy to approximately the normal length of pregnancy in the rat,³⁸ and (2) histochemical studies on these corpora lutea indicate that luteal life is prolonged as a result of the induced decidual tissue.¹¹ On the other hand, Bryans' work⁷ and Hisaw's

observations²¹ indicate that HCG, a hormone of the placenta, will prolong luteal life in monkeys. Inasmuch as LtH does not possess the ability to prolong the physiological aspects of the primate corpus luteum, it seems entirely justifiable to assign a key role to HCG, during early pregnancy at least.²¹ As a matter of fact, Hartman^{19, 20} and Hisaw²¹ have discussed the theory that the corpus luteum normally undergoes involution at about the twenty-fifth day in every normal pregnant monkey. Furthermore, at this time, the definitive vascular relationship between mother and fetus is fairly well established, and the destruction of maternal tissue by the invading trophoblast has decreased noticeably. This is about the time the chorionic gonadotropin disappears from, or is barely detectable in, the urine of the pregnant monkey.^{19, 20}

In view of the previously mentioned statements regarding the role of the placenta in pregnancy, it seems of more than academic interest to endeavor (1) to relate some of the recently acquired information to our understanding of the endocrine physiology of reproduction, and (2) to attempt to relate the action of the cytotrophoblast with the syncytial trophoblast.

Early placental formation and development in the macaque monkey have been divided into three stages by Wislocki and Streeter:⁴⁰ the first, occurring between the ninth and tenth days, involves the implantation of the free blastocyst and the invasion of the trophoblastic plate into the uterine epithelium; the second, taking place between the eleventh and fifteenth days, characterizes the differentiation of the solid trophoblastic plate into the syncytial trophoblast with lacunae containing maternal blood; and the third, commencing on approximately the fifteenth day and lasting through the thirty-fifth day, delineates the development of chorionic villi and the resulting formation of the definitive placenta.⁴⁰

There are now numerous facts, all firmly established, that relate these placental changes with the endocrine physiology of the monkey.

The first is identical with the luteal aspect of the menstrual cycle, and it is the LtH that brings about progesterone secretion from the corpus luteum.

By the end of the tenth day implantation of the ovum has fully taken place, and the second stage is characterized by the appearance of chorionic luteotropin elaborated by the maturing trophoblast. Placental luteotropin adopts the previous adenohypophyseal LtH function, thus preventing involution of the corpus luteum. The latter normally occurs at the expected time of menstruation when the corpus luteum remains under the sole control of LtH.

The second stage climaxes with the further development and transformation of the corpus luteum spurium into the corpus luteum verum (of pregnancy), and the concomitant reduction and cessation of the secretion of progesterone by the luteal tissue. Therefore, the third stage of pregnancy in the primate (macaque) commences with the loss of corpora luteal function, and continues to do so until parturition. Consequently, the placenta must be essentially an endocrine organ of pregnancy.²¹

Furthermore, and only quite recently, Smith³⁰ in a series of experiments on monkeys has shown that pregnancy continues after hypophysectomy in the monkey.

Finally, it should be emphasized that most of the available evidence points to the placenta as the dominant endocrine organ of gestation, for it can adopt both adeno-hypophyseal and ovarian functions. Hartman demonstrated that the corpus luteum is dispensable by the twenty-fifth day,^{19, 20} and van Wagenen and her associates^{13, 34} showed clearly that the placenta will remain until term and that it will continue to elaborate estrogen in the absence of both ovaries and fetus. Here we begin to see some of the missing links of the evolution of viviparity fall into what is envisaged as an orderly sequence of events in that magnificent accommodation of nature from oviparity to viviparity.^{2, 21}

In tracing through certain of the recently accumulated data and the experiments by Hartman,^{19, 20} Hisaw,²¹ and van Wagenen,^{13, 34} one question looms sharply: What is the relationship between the cytotrophoblast (the source of HCG and possibly other proteinaceous tropic hormones) and the syncytial trophoblast (placental tissue source of estrogen(s), progesterone, and possibly other steroids, including a large number of 17-ketosteroids)? Unfortunately, there are no available data on this point. Admittedly, it is tempting to speculate on a so-called feedback system resident between the cytotrophoblast and the syncytium, much as one would like to envisage for the pituitary and its several target organs. This is as far as speculation can go in view of the present status of our knowledge.

SUMMARY AND CONCLUSIONS

The ovaries of intact, immature albino rats given HCG were enlarged and showed follicular growth, ovulation, and corpus luteum formation.

HCG repairs and stimulates ovarian interstitial tissue in hypophysectomized, immature rats.

Evidence is offered showing that HCG synergizes with adeno-hypophyseal FSH.

HCG induces ovulation and prolongs pseudopregnancy in adult, albino rats.

Aminopterin, or 4-amino-PGA, can interfere with the action, or the actions of, small doses of HCG on the sex tract and adrenal glands, but not on the thyroid or pituitary glands of intact, immature rats.

These results and those obtained by others are used as a point of departure for a commentary on the evolution of viviparity and the role of the placenta and HCG in the adoption of ovarian and pituitary function during pregnancy in the primate.

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I acknowledge the supply of human chorionic gonadotropic hormone (Antuitrin-S) from Parke, Davis and Company, and of the 4-amino-PGA (aminopterin) from the Lederle Laboratories for these experiments.

REFERENCES

1. ALBERT, A. & W. L. MONEY. 1946. The comparative effect of human chorionic and equine gonadotrophins on the rate of ovarian growth. *Endocrinology*. **38**: 219-229.

2. AMOROSO, E. C. 1955. Endocrinology of pregnancy. *Brit. Med. Bull.* **11**: 117-125.
3. ASCHHEIM, S. & B. ZONDEK. 1927. Hypophysenvorderlappenhormon und Ovarialhormon im Harn von Schwangeren. *Klin. Wochschr.* **6**: 1322.
4. BARDAWIL, W. A., A. T. HERTIG & J. T. VELARDO. 1957. Regression of trophoblast. I. Hydatidiform mole; a case of unusual features, possible metastasis and regression; review of literature. *Obstet. & Gynecol.* **10**: 614-625.
5. BEHRMAN, S. J. & P. NIEMAN. 1955. Serum gonadotrophins in normal and abnormal pregnancy. II. Function and action of chorionic gonadotrophin hormones. *Fertility and Sterility*. **6**: 415-420.
6. BRADBURY, J. T., W. E. BROWN & L. A. GRAY. 1950. Maintenance of the corpus luteum and physiologic actions of progesterone. *Recent Progr. in Hormone Research*. **5**: 151-194.
7. BRYANS, F. E. 1951. Progesterone of the blood in the menstrual cycle of the monkey. *Endocrinology*. **48**: 733-740.
8. BURDICK, H. O. & M. CRUMP. 1951. The effect of induced ovulation on pregnancy. *Endocrinology*. **48**: 273-276.
9. BURROWS, H. 1949. *Biologic Actions of Sex Hormones*. : 15-16. Cambridge Univ. Press. Cambridge, England.
10. D'AMOUR, F. E. & M. C. D'AMOUR. 1940. The biologic potency of international standard chorionic gonadotropin. *Endocrinology*. **26**: 93-96.
11. DAWSON, A. B. & J. T. VELARDO. 1955. A histochemical study of lipids of the corpora lutea of the rat during pseudopregnancy. *Am. J. Anat.* **97**: 303-329.
12. DICZFALUSY, E. 1953. Chorionic gonadotrophin and estrogens in the human placenta. *Acta. Endocrinol. Suppl.* **12**(xii): 1-175.
13. DORFMAN, R. I. & G. VAN WAGENEN. 1941. The sex hormone excretion of adult female and pregnant monkeys. *Surg. Gynecol. & Obstet.* **73**: 545-548.
14. EMMENS, C. W. 1938. Analysis of the assays carried out in various laboratories on the contributions offered towards the international standard preparation of the gonadotrophic substance of urine of pregnancy. *Bull. Health Org. League of Nations*. **8**. Extract No. **15**: 862-883.
15. ENDERS, A., J. T. VELARDO, A. B. DAWSON & A. G. OLSEN. 1953. The influence of chorionic gonadotropic hormone on the length of pseudopregnancy in the rat. *Anat. Record*. **117**: 534-535.
16. FLUHMAN, C. F. 1951. Augmentative effects of steroids and chorionic hormone on rat ovary. *Proc. Soc. Exptl. Biol. Med.* **77**: 472-473.
17. HALBAN, J. 1905. Die innere Secretion von Ovarium und Placenta und ihre Bedeutung für die Function der Milchdrüse. *Arch. Gynaekologie*. **75**: 353-441.
18. HARRISON, R. G. 1956. The effect of prolonged injections of chorionic gonadotrophin on the rat testis. *Rept. Brit. Fertility Assoc.*
19. HARTMAN, C. G. 1939. Non-effect of ovariectomy during pregnancy in the rhesus monkey. *Am. J. Obstet. Gynecol.* **37**: 287-290.
20. HARTMAN, C. G. 1941. Non-effect of ovariectomy on the twenty-fifth day of pregnancy in the rhesus monkey. *Proc. Soc. Exptl. Biol. Med.* **48**: 221-223.
21. HISAW, F. L. 1944. The placental gonadotrophin and luteal function in monkeys (*Macaca mulatta*). *Yale J. Biol. Med.* **17**: 119-137.
22. HOBSON, B. M. 1953-1954. The routine diagnosis of pregnancy, hydatidiform mole and chorionepithelioma using female *Xenopus laevis*. *Trans. Edinburgh Obstet. Society Session*. **106**: 33-38.
23. LEVIN, L. 1944. *Chemistry and Physiology of Hormones*. : p. 162. Amer. Assn. Adv. Sci., Washington, D. C.
24. LORAIN, J. A. 1957. Some general principles in the bioassay of anterior pituitary and placental hormones in blood with special reference to clinical problems. *In Hormones in Blood, Ciba Fdn. Colloq. Vol. XI*: 19-37. G. E. W. Wolstenholme and Elaine C. P. Millar, Eds. Little, Brown, Boston, Mass.
25. MADDOCK, W. O. & W. O. NELSON. 1952. The effects of chorionic gonadotropin in adult men: increased estrogen and 17-ketosteroid excretion, gynecomastia, Leydig cell stimulation and seminiferous tubule damage. *J. Clin. Endocrinol. Metabolism*. **12**: 985-1014.

26. NEWTON, W. H. 1938. Hormones and the placenta. *Physiol. Revs.* **18**: 419-446.
27. NOBLE, R. L. & E. R. PLUNKETT. 1955. Biology of the gonadotrophins. *Brit. Med. Bull.* **11**: 98.
28. ROSENFELD, S., B. LAPAN & H. BARRON. 1941. Effects of human pregnancy serum on parturition in the rat. *Endocrinology.* **29**: 276-280.
29. SEGAL, S. J. 1957. Response of the weaver finch to chorionic gonadotropin and hypophysial luteinizing hormone. *Science.* **126**: 1242-1243.
30. SMITH, P. E. 1954. Continuation of pregnancy in rhesus monkeys (*Macaca mulatta*) following hypophysectomy. *Endocrinology.* **55**: 655-664.
31. SOHVAL, A. R. 1958. The anatomy and endocrine physiology of the male reproductive system. Chap. VII. *In* The Endocrinology of Reproduction. p. 247. Joseph Thomas Velardo, Ed. Oxford Univ. Press. New York, N. Y.
32. STEELMAN, S. L. & F. M. POHLEY. 1953. Assay of the follicle stimulating hormone based on the augmentation with human chorionic gonadotropin. *Endocrinology.* **6**: 604-616.
33. STEWART, H. L., JR., M. E. SANO & T. L. MONTGOMERY. 1948. Hormone secretions by human placenta grown in tissue culture. *J. Clin. Endocrinol.* **8**: 175-188.
34. VAN WAGENEN, G. & W. H. NEWTON. 1943. Pregnancy in the monkey after removal of the fetus. *Surg. Gynecol. & Obstet.* **77**: 539-543.
35. VELARDO, J. T. 1952. Effects of growth inhibitors on response of rat's uterus to estrogen. *Proc. Soc. Exptl. Biol. Med.* **78**: 872-874.
36. VELARDO, J. T. & F. L. HISAW. 1953. Inhibitory effects of Aminopterin on decidual development. *Am. J. Physiol.* **172**: 535-540.
37. VELARDO, J. T. & F. L. HISAW. 1953. Inhibition of the Hooker-Forbes response by Aminopterin. *Proc. Soc. Exptl. Biol. Med.* **81**: 652-654.
38. VELARDO, J. T., A. G. OLSEN, F. L. HISAW & A. B. DAWSON. 1953. The influence of decidual tissue upon pseudopregnancy. *Endocrinology.* **53**: 216-220.
39. VELARDO, J. T. 1958. *The Endocrinology of Reproduction.* : 101-212. Oxford Univ. Press. New York, N. Y.
40. WISLOCKI, G. B. & G. L. STREETER. 1938. On the placentation of the Macaque (*Macaca mulatta*) from the time of implantation until about the formation of the definitive placenta. *Carnegie Inst. Wash., Publ. No.* **496**: 1-66.

Discussion of the Paper

P. TROEN (*Beth Israel Hospital, Boston, Mass.*): The endocrine function of the human placenta has been under study in my laboratory for several years, utilizing the technique of perfusion of the intact freshly obtained gland. In view of J. T. Velardo's remarks, several of my findings are of interest. E. E. Gordon and I have noted that the addition of human chorionic gonadotropin (HCG) together with estradiol causes a marked stimulation of citrate utilization: an energy-yielding process. Estradiol alone does not do this in the perfused gland (in contrast to the findings of others in placental homogenates) and HCG alone causes only a slight increase of citrate utilization.¹

I have found that another effect of HCG on the perfused human placenta is on estrogen metabolism.² Under the influence of HCG, radioactive estradiol is converted not only to estrone but also to a third substance with the characteristics of estriol. Without HCG, only estrone is produced. Estradiol conversion to estriol has not been shown previously in human placental tissue.

These two findings suggest strongly that the trophoblast is not only autonomous but also self-regulatory, at least for some hormonal processes.

Finally, it should be noted that it has long been suggested that the human placenta can take over not only ovarian and pituitary function but also

adrenocortical function. Our studies² on corticosteroid production by the perfused human placenta indicate that such a potentiality is indeed present.

References

1. TROEN, P. & E. E. GORDON. 1958. I. Perfusion studies of the human placenta. I. Effect of estradiol and human chorionic gonadotropin on citric acid metabolism. *J. Clin. Invest.* **37**: 1516.
2. TROEN, P. 1959. Perfusion studies of the human placenta. *In* Conf. on Recent Progress in Endocrinology of Reproduction. Academic Press. New York, N. Y.

CARL G. HARTMAN (*Ortho Research Foundation, Raritan, N. J.*): If we review the chronological stages of human development as seen in the Hertig-Rock¹ series of ova to cover the first 12 days (FIGURE 1), it is evident that in

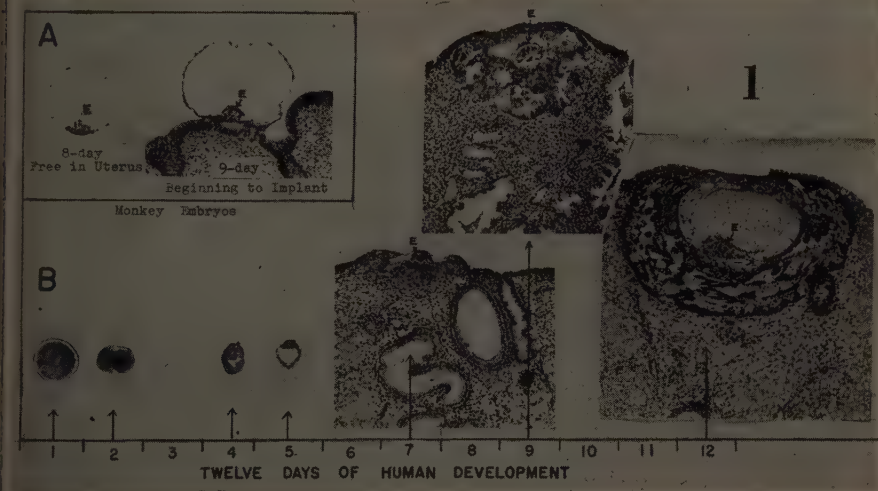


FIGURE 1. All of the eggs and embryos are reproduced on the same scale.¹ It is to be noted that, while the embryo itself has differentiated by the twelfth day, it has increased but little in bulk.

the first 5 days there have been only cellular division and differentiation, but no actual growth in size. At 7 days the human vesicle (blastocyst) embeds. A tremendous activity of the trophoblast that eats its way into the endometrium begins. On the twelfth day there is a comparatively large structure of typical primate placental architecture. Actually, the structure is not much larger than the head of a pin, but already it is giving off chorionic gonadotropin into the blood stream. The ensemble is called, in German, *das Ei*. It should be noted that the embryo itself thus far has undergone but little growth; it is practicing good logistics in getting the commissary organized in preparation for its own spurt of growth.

All mammals pass through a vesicular stage. In the monkey the blastocyst makes contact with the uterine epithelium on the ninth day, and its effect on the epithelial cells is apparent in a few hours. In the monkey the

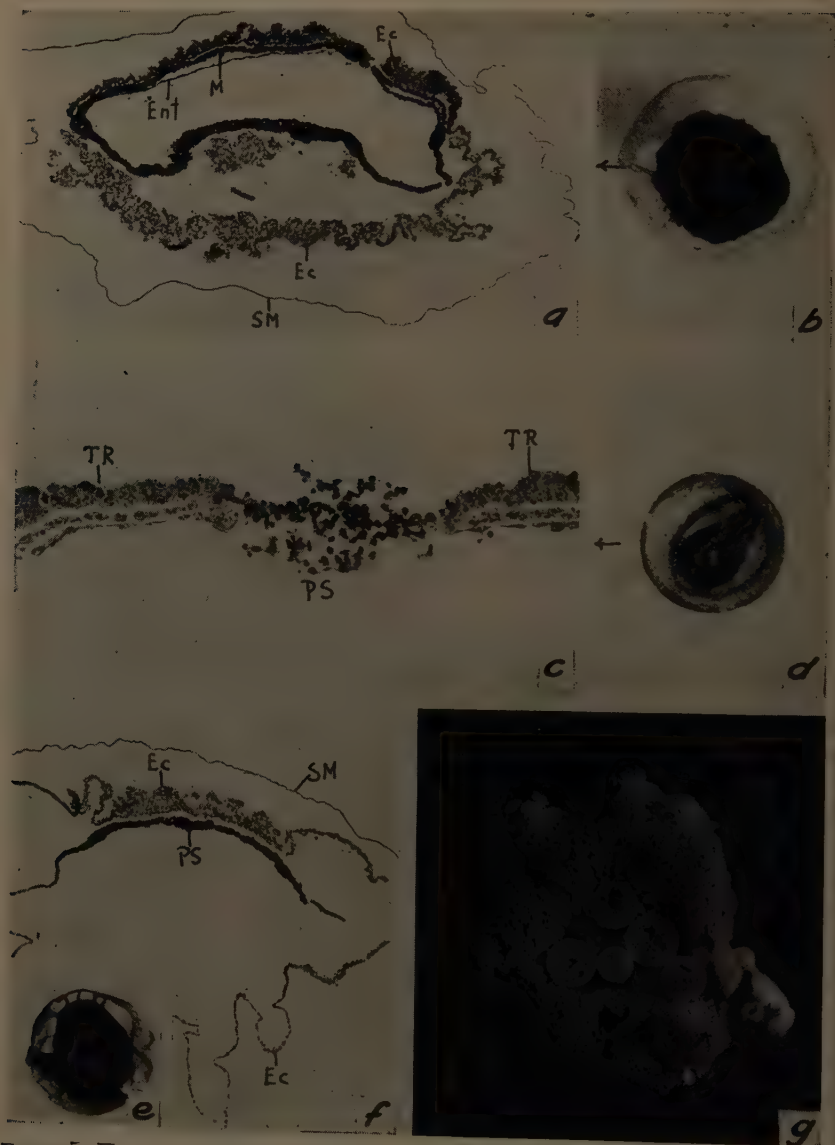


FIGURE 2. (g) An abnormal batch of opossum eggs photographed alive in the open uterus; only the largest vesicle was normal. (a and f) Ec represents excessive proliferation of ectoderm cells (neoplastic?); Ent, entoderm; M, mesoderm; SM, shell membrane, a character that marsupials share with their reptilian ancestors; (c) PS, degenerating embryo in the primitive streak stage; (b, c, and d) eggs photographed *in toto* after fixation.

antiembryonic and the embryonic zones are equally capable of stimulating the pregnancy reaction (a placental plaque of the uterine epithelium, not a decidual reaction as in man). Hence the monkey develops a double placenta, a primary placenta, and a secondary one, with intercommunicating blood vessels.

In some rodents the sticky antiembryonic pole attaches temporarily; this attachment degenerates after the permanent attachment is effected at the opposite pole. In the opossum, the relationship of the chorion to the uterine epithelium is striking. At the time of ovulation the epithelial lining is thick and its nuclei pseudostratified. Late in the 13-day gestation period of the opossum the epithelial lining thins considerably and the chorionic epithelium rests in immediate contact with it (see Figures 26 and 27 in Hartman² and Figure 8 in Hartman³). This arrangement is effective for nourishment of the embryo up to 12 $\frac{3}{4}$ days. The hamster fetus, born on the sixteenth day, requires a rather complicated placenta; such is the difference in 3 days of embryonic development.

FIGURE 2 shows 2 opossum vesicles from the same batch in which there is a peculiar hyperplasia of ectodermal cells. There is considerable mitotic activity, every third cell being in division instead of approximately 1 in 20. Can this represent, in the primitive streak of the opossum, the equivalent of a neoplasm? Embryonic neoplasms are described elsewhere in this monograph.

References

1. HERTIG, A. T., J. ROCK & C. E. ADAMS. 1956. Am. J. Anat. **98**: 435-494.
2. HARTMAN, C. G. 1923. The oestrous cycle of the opossum. Am. J. Anat. **32**: 353-421.
3. HARTMAN, C. G. 1927. Some cases of defective *Corpora lutea* correlated with pathological uteri and death of embryos. Contrib. to Embryol. Carnegie Inst. Wash. **19**: 285-300.
4. RAFFERTY-MACHLIS, G. & C. G. HARTMAN. 1953. Early death of the ovum of the opossum with observations on moribund mouse eggs. J. Morphology. **92**: 455-486.

C. L. BUXTON (*Yale University School of Medicine, New Haven, Conn.*): I was interested in C. G. Hartman's remarks about the logistics of the trophoblast because it does seem that this minute structure has an extraordinary responsibility shortly after the time of implantation of the ovum. Apparently during the few days thereafter the trophoblast has not only the responsibility of appropriately embedding itself in the maternal endometrium but also of producing a substance that will so stimulate the maternal corpus luteum that the latter, in turn, is induced to secrete a hormone that will so adequately support the decidua that this also, in turn, will remain a good nidation site for the trophoblast. This requirement of producing a hormone to maintain its own salvation, so to speak, which nature has placed on the trophoblast, seems to me to be one of the severest requirements of nature, and it therefore is not unreasonable to think that there must be many occasions when these logistics are not fulfilled and the new trophoblast and its attendant embryo cannot provide themselves a good nidation site in which to flourish.

Part II. Hydatidiform Mole

GENESIS OF HYDATIDIFORM MOLE: OLD AND NEW CONCEPTS

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Hydatidiform mole (HM) is defined as a conceptus, usually devoid of an intact fetus, in which all or many of the chorionic villi show (1) gross nodular swelling culminating in cyst formation, (2) disintegration of blood vessels, and (3) variable proliferation of trophoblast. Subsequent discussion of the genesis of HM will be based on this definition. Genesis will be considered to include both formal genesis and causal genesis; both pathogenesis and etiology.

It has long been recognized¹ that many abnormal, young, aborted conceptuses show an alteration of their chorionic villi quite similar to that of the classical HM, a disturbance commonly termed hydatidiform degeneration. Affected chorionic villi are miniature replicas of the larger, grapelike vesicles of HM, hardly differing from the latter except in size. Hydatidiform degeneration of young villi may easily be overlooked unless the specimen is examined histologically or under a dissecting microscope, while the bulbous swellings of HM are large enough to be obvious in gross.

Occasionally a conceptus is observed with villar distortion intermediated between that of hydatidiform degeneration and that of HM. Hertig and Li designated such an intermediate form as a transitional mole, defining it as "an abortus in which the various portions of the ovum (chorion, villi, amnion, and embryo) can still be identified but in which enough villi have sufficient hydatidiform degeneration to make the diagnosis clearly evident from the gross specimen."²

In the material that we reported, spontaneously aborted, pathological ova with hydatidiform degeneration of their chorionic villi showed a mean menstrual age of 10.2 weeks, while transitional moles averaged 16.6 weeks and cases of typical HM averaged 17.4 weeks. Furthermore, in our series the histological changes manifest in villi of transitional moles tended to be intermediate between those of the ova showing hydatidiform change and those of HM. We therefore concluded that the three categories constituted an actual pathogenetic series, representing successive stages in the development of a villar abnormality. We considered HM to be a delayed abortion of a pathological conceptus retained *in utero* for a sufficient time to allow full development of the swelling of the villi, swelling that could have progressed only to the stage of hydatidiform degeneration or of transitional mole had the pregnancy terminated at an earlier date.

The following data, based on material examined in the Laboratory of Pathology, Boston Lying-In Hospital, Boston, Mass., have not been published previously. Evidence of the continuity of this presumed morpho-

genetic series has been obtained by measurement of villi of conceptuses representing the various stages in the series.

Individual villi were selected at random from frondosal areas of intact chorionic sacs of abortuses, 36 of which showed hydatidiform change, while 61 appeared normal (diagnosis confirmed histologically). Individual villi were also selected at random from 12 transitional moles and 45 HM. Each villus was placed on a half-millimeter-grid background and carefully delineated in outline, using a dissecting microscope. Measurements of the maximal diameter of bulbs, or expanded segments of villi, and of the minimal diameter of stalks, or narrow segments of villi, were then obtained from the drawings. Measurements of at least 5 bulbs and 5 stalks were made for each specimen examined, and mean values were then computed for bulb and stalk diameters of each specimen.

It was found that bulb diameter and stalk diameter vary concurrently with age of the abortus. In FIGURE 1, the ratios of mean bulb diameter to mean stalk diameter are plotted on a logarithmic scale against the corresponding determinations of menstrual age of the abortus (as obtained from the clinical histories of the patients), plotted arithmetically. It will be seen that there are apparently two growth series diverging from a common point of origin: first, normal villi and, second, a composite sequence of villi showing hydatidiform change, villi of transitional moles, and villi of HM.

Besides demonstrating continuous variation from hydatidiform degeneration to HM, FIGURE 1 shows that normal villi become somewhat more evenly cylindrical with age, as indicated by slight diminution in the bulb/stalk (B/S) ratio, while villi in the abnormal series manifest marked progression in the disparity between diameters of their bulbs and stalks—progressive swelling of vesicles—as indicated by increase of the bulb/stalk ratio with age.

In any semilogarithmic plot, straight lines indicate constant rate of change. The greater the slope of a line, referred to the logarithmic axis of plotting, the less will be the rate of change. Since in FIGURE 1 the line of trend along which the values for abnormal villi are distributed shows increasing slope, referred to the B/S axis, with increasing duration of pregnancy, it may be inferred that hydatidiform degeneration progresses more rapidly earlier in pregnancy than it does later in pregnancy.

The values for the 2 series of villi plotted in FIGURE 1 show divergence from a common point at approximately $7\frac{1}{2}$ weeks, menstrual age. This may be considered an average time when hydatidiform change first becomes evident in gross, although transition from normal villus to hydatidiform villus might take place at any time from the fifth through the tenth week. This critical epoch may then be regarded as the teratogenic termination period, using the term as defined by Schwalbe,³ of the abnormal growth that leads from hydatidiform alteration of villi to HM.

Histologically, "the loose villous stroma of hydatidiform degeneration is merely an accentuation of the normally loose mesenchyma (or connective tissue) present in the early chorionic villus of the ovum of approximately three-weeks' ovulation age or five-weeks' menstrual age."² Subsequent progression of the histological changes leading to HM includes disintegration

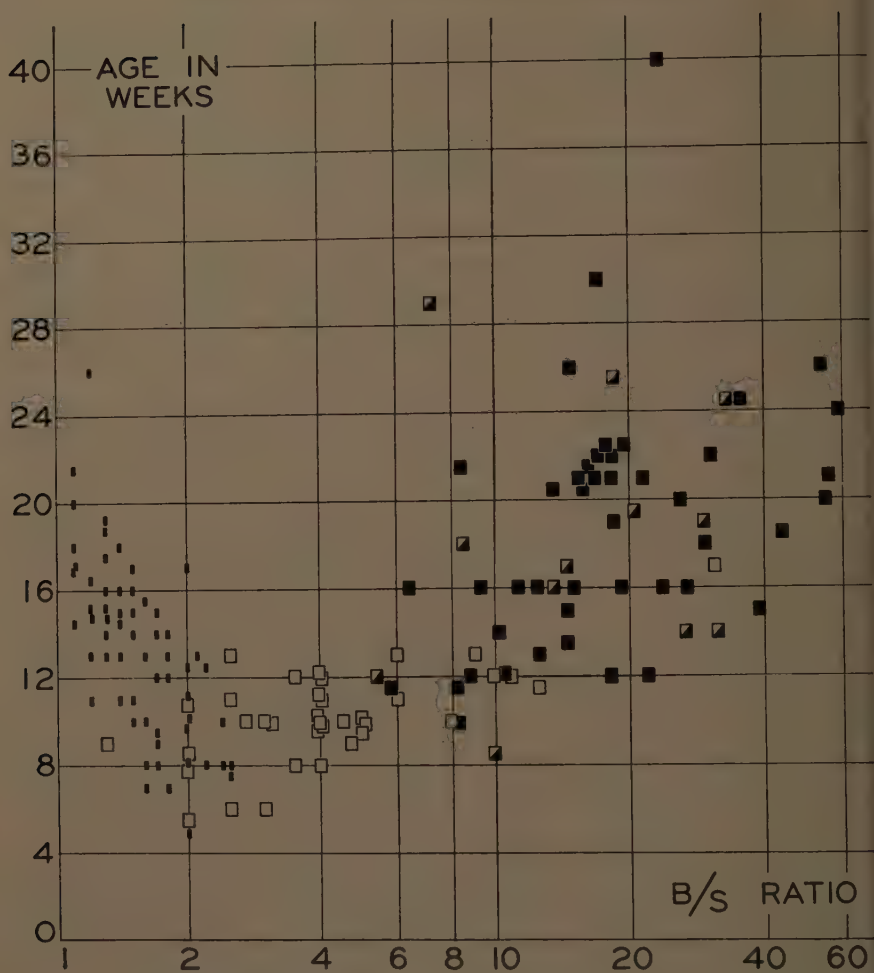


FIGURE 1. Progressive development from hydatidiform degeneration of early chorionic villi through transitional moles to mature HM. The ratio of the mean maximal diameter of bulbs (expanded segments of villi) to the mean minimal diameter of stalks (narrow segments of villi) is plotted against the corresponding gestational age for each conceptus studied. HM are plotted as solid squares, transitional moles as diagonally divided squares, villi showing hydatidiform degeneration as open squares, and normal villi (for comparison) as small solid bars. Note semilogarithmic scale.

of the stroma of the swollen portions of the villi, with compression of the disordered stroma into a shell that surrounds a central empty space which, in histological sections, corresponds to central accumulation of fluid.

Fluid aspirated from living vesicles of HM has been subjected to careful chemical analysis by McKay *et al.*^{4, 5} for comparison with fluid from normal chorionic sacs and with maternal serum (TABLE 1). Fluids from chorionic sacs and from HM vesicles tend to show similar deviations from maternal

serum in regard to concentration of various substances analyzed. From the standpoint of electrolyte composition, HM fluid is similar to other interstitial fluids as found in ascites and edema.

The osmotic activity of fluid from HM is found to be lower than that of serum, so that water is retained (and indeed accumulates) within HM villi against the force of a higher osmotic pressure in the maternal serum that surrounds these vesicles *in utero*. Osmosis, therefore, cannot be the mechanism of villar swelling. It may be presumed that the vesicles of HM accumulate fluid through continued secretion by the trophoblast into villi that are unable to transport away such fluid, due to deficiencies in their vasculature.

TABLE 1
CHEMICAL ANALYSES*

Constituent	HM vesicle fluid	Chorionic fluid	Maternal serum
Sodium.....	123.0 mEq./l.	132.2	136.0
Potassium.....	6.7 mEq./l.	5.3	5.0
Chloride.....	107.0 mEq./l.	113.2	115.0
Total protein.....	1.07 gm. %	0.733	6.1
Total solutes.....	0.254 osmoles/l.	not determined	0.275
Glucose.....	72.5 mg. %	89.0	118.7
Calcium.....	7.4 mg. %	not determined	9.1
Inorganic phosphate.....	12.0 mg. %	9.06	6.9
Phosphatase (Bodansky)			
Alkaline.....	8.4 U.	4.85	2.6
Acid.....	7.5 U.	2.56	0.4

* McKay *et al.*^{4, 5}

Disintegration and ultimate disappearance of blood vessels from villi already have been indicated as essential features in the definition of HM. It remains to state that not only villi of transitional moles, but also villi of early hydatidiform degeneration show corresponding discontinuity or fragmentation of blood vessels.² It is known⁶ that the blood vessels of normal chorionic villi are both autochthonous and autonomous, arising independently of the development of a vascular system within the embryo. Normally, blood vessels in the villi join those of the embryo in a functioning circuit at a menstrual age of five weeks. Absence of an intraembryonic vascular system at this critical period, due either to nondevelopment of an embryo or to previous death of the embryo, effectively prevents further development of functioning vessels within the villi.

The third feature of HM recognized in the definition initiating this discussion is the proliferation of trophoblast. It would appear that there is both relative and absolute increase of trophoblast on the surface of villi during the development of HM, inasmuch as, mathematically, the surface area of any expanding spheroid increases as a function of the second power of the mean diameter, and HM vesicles are recognized as showing both a

general coating of trophoblast approximately similar in depth to that over normal villi, as well as focal thickenings of this layer. There have been, however, no morphologic quantitations of the extent of this trophoblastic overgrowth. The well-known excess production of chorionic gonadotrophin in molar pregnancy may be due, in part, to increased *amount*, and in part to augmented *function*, of trophoblast. Altered function of trophoblast is implied by histochemical studies that have shown deficiencies of iron, calcium, and acid phosphatase in trophoblast of HM villi,⁷ while these cells appear to show excesses of glycogen⁷ and of Schiff-positive material interpreted as gonadotropin.⁸

Histological differences have been made the basis of schemes^{9, 9a, 10} for prognostic grading of HM in attempts to predict the likelihood of neoplastic transformation and propagation of the trophoblast as choriocarcinoma. The evaluation of this controversial subject lies beyond the scope of this paper; but it may be pointed out that none of the cytological criteria employed in the various systems of grading correlate well with size of the molar villi, that is, none of these criteria are directly related to molar swelling: "... early and intermediate stages in the formation of HM show little alteration of trophoblastic histology or pattern."^{9a}

Contrary to the theory of formal genesis embodied in the foregoing discussion, overgrowth of trophoblast has been considered¹¹ of primary importance in production of HM. Vigorous proliferation of trophoblast is admittedly a feature of any normal conceptus at the time when altered growth of villi leading to HM begins; in the present state of our knowledge it is not possible to exclude the existence of small excesses in early molar pregnancy. Nevertheless, evidences of either overgrowth or qualitative abnormality of trophoblast *early* in the development of HM have not been produced, and one does not need to postulate primary action of trophoblast in order to arrive at a working theory of molar genesis.

Causal genesis, or etiology, of HM will now be considered. In the search for distinguishing characteristics of pregnancies terminating in HM, maternal age has long^{1, 12} been under suspicion. In an attempt to bring this question to a definitive solution, a representative (but not exhaustive) compilation has been prepared, assembling data on age incidence of HM as reported in the literature.

Each series chosen for inclusion in this study is purportedly an original, consecutive sample of molar pregnancies collected in a specific place at a specific time. It is believed that there is no duplication of cases, and no intentional bias of selection. It is, of course, impossible to exclude unintentional bias, such as that which might occur, for example, if there were a tendency for relatively more young women than old to enter hospital for delivery, molar or otherwise, or if older women were more apt to experience adverse symptoms from molar pregnancy than younger women, and hence would be more likely to be hospitalized. Only European and American series have been considered, in view of recent suspicion that HM may occur with a different incidence in eastern Asia.

For purposes of comparison, an appropriate control or normal popula-

tion representing all births has been selected for each of the series of molar pregnancies to be considered, utilizing published international vital statistics.¹³⁻¹⁵ Each control population is drawn from the country in which the series of HM was collected and at as nearly the same period of time as conveniently possible.

In TABLE 2, data of each series of HM pregnancies, expressed in terms of the percentages of cases occurring in each of four age groups, are compared with corresponding percentages of all pregnancies occurring in the same age groups in the normal population taken for control.*

Percentile deviations are obtained by subtracting algebraically the percentage value for normal population from the percentage value for HM in each age category for each series. Positive values indicate an excess of HM cases over expectancy based on the normal comparison population, and negative values indicate a corresponding deficit.

It is observed that deviations show a consistent trend throughout the various series. HM is shown to occur more frequently than expected in mothers 19 years of age or younger, as well as in mothers 40 years of age or older. Corresponding deficits in observed incidence of HM are noted in the middle range, extending from age 20 years through age 39 years.

At the end of TABLE 2 the data are summarized by a statement of the totals of the HM cases in the respective age groups in all series, expressed as percentages based on the grand total of 1378 HM. The percentages in each age group in each control series have been multiplied by the percentage of cases which the particular HM series contributes to the grand total, and these terms have then been added to arrive at a general, weighted control population for comparison.

As in the case of the individual series, the summary compilation indicates that HM is relatively more common in the younger and older age ranges. Appropriate Chi-square testing has shown that these differences are statistically significant.

It is of interest to compare the age incidence of HM with those of several other types of unusual birth products that have been claimed³⁵⁻³⁸ to show an age influence (TABLE 3). Mongoloids appear much less frequently in the age range 20 to 29 years and much more frequently after age 39. Abortions, congenital malformations of all types, and dizygotic twins all show a tendency to appear with greater frequency in the age category of 30 to 39 years.

The comparative age incidence of HM and other unusual birth products is presented graphically in FIGURE 2. Here vertical bars correspond to the excess or deficit of molar (or other abnormal) births compared to the pro-

* Throughout this compilation, the relatively few cases of pregnancies (normal or molar) reported as of unknown age have been excluded both from individual categories and from the totals.

Some of the series of HM cases were originally reported in a slightly different scheme of age categories, such as 20 years and under and 21 years to 30 years. In a few cases, these categories have been taken over directly, ignoring the negligible error that is thus introduced into the compilation. Where, however, authors have also supplied ages of the individual HM cases, new tabulations have been constructed, employing the standard categories of TABLE 2.

TABLE 2
AGE INCIDENCE OF HM: DATA FROM LITERATURE

Source	Percentages of series in each age group				Number of cases in series
	19 yrs. & younger	20-29	30-39	40 yrs. & older	
United States ¹⁶⁻¹⁸	15.5	57.6	18.2	8.5	528
Control: USA, 1936.....	12.6	57.6	26.1	3.6	
Deviation.....	+ 2.9	0	- 7.9	+ 4.9	
Chile ¹⁹	16.0	45.6	28.8	9.4	94
Control: Chile, 1941.....	9.4	48.2	34.3	8.0	
Deviation.....	+ 6.6	- 2.6	- 5.5	+ 1.4	
Czechoslovakia ²⁰	9.1	31.8	31.8	27.2	22
Control: Czech., 1925-1928.....	5.3	59.2	31.0	4.4	
Deviation.....	+ 3.8	-27.4	+ 0.8	+22.8	
Dominican Republic ²¹	20.0	46.6	33.4	0	15
Control: Dominican Repub., 1944....	11.4	55.6	27.0	5.9	
Deviation.....	+ 8.6	- 9.0	+ 6.4	- 5.9	
France ²²	8.7	60.9	17.4	13.0	23
Control: France, 1954.....	3.7	62.6	29.4	4.2	
Deviation.....	+ 5.0	- 1.7	-12.0	+ 8.8	
Italy ²³	7.1	32.2	30.4	30.4	56
Control: Italy, 1936.....	2.6	51.9	38.4	6.9	
Deviation.....	+ 4.5	-19.7	- 8.0	+23.5	
Germany ²⁴⁻²⁸	6.4	43.2	31.4	19.1	220
Control: Germany, 1937.....	2.8	53.8	38.8	4.7	
Deviation.....	+ 3.6	-10.6	- 7.4	+14.4	
England ^{29, 30}	4.2	37.9	26.3	31.6	95
Control: England, 1921.....	3.2	51.4	41.3	3.9	
Deviation.....	+ 1.0	-13.5	-15.0	+27.7	
Netherlands ³¹⁻³³	5.2	48.5	21.6	24.6	134
Control: Netherlands, 1936.....	2.3	47.8	42.9	7.1	
Deviation.....	+ 2.9	+ 0.9	-21.3	+17.5	
Norway-Sweden ³⁴	3.7	35.1	34.0	27.2	191
Control: Norway, 1910-1911.....	2.0	43.2	43.6	11.2	
Deviation.....	+ 1.7	- 8.1	- 9.6	+16.0	
All HM series combined.....	10.2	47.6	25.0	17.2	1378
Weighted control population.....	7.0	52.9	34.5	5.6	
Deviation.....	+ 3.2	- 5.3	- 9.5	+11.6	

portion of all births in the corresponding age groups of the general population. As in TABLE 3, data are stated in terms of percentages, the 4 age groups of each series comprising 100 per cent.

It is regretted that data are not at hand for direct exclusion of the possibility that gravidity or paternal age (both correlates of maternal age) lead to excess production of HM in both the youngest and oldest age categories, rather than maternal age itself. Assuming tentatively that the age influence is, at least principally, a matter of maternal age, one can easily suggest hypothetical mechanisms through which this factor may operate. Reproductive efficiency may be influenced by deficiencies of either endometrium, ovaries, or total hormonal status of the mother. Any of these could be relatively less adequate during both the earlier and later years of the reproductive period, and might lead to debility or death of the embryo, with subsequent deviation of villar development toward HM.

TABLE 3
AGE INCIDENCE OF VARIOUS ABNORMAL BIRTH PRODUCTS

Series	Percentages of series in each age group				Number of cases in series
	19 yrs. & younger	20-29	30-39	40 yrs. & older	
Mongoloids ³⁵	3.6	25.6	42.2	28.7	2738
Malformations ³⁶	7.1	55.8	32.5	4.6	607
Abortions ³⁷	1.8	45.6	43.9	8.6	2124
Dizygotic twins ³⁸	3.8	47.8	42.1	6.3	1112
Control for all series; U. S. A., 1936...	12.6	57.6	26.1	3.6	
Deviations for series					
Mongoloids.....	- 9.0	-32.0	+16.1	+25.1	
Malformations.....	- 5.5	- 1.8	+ 6.4	+ 1.0	
Abortions.....	-10.8	-12.0	+17.8	+ 5.0	
Dizygotic twins.....	- 8.8	- 9.8	+16.0	+ 2.7	

Molar multiparity has suggested an infectious etiology. Positive serologic reactions for toxoplasmosis have been reported³⁹ in a patient who experienced four consecutive molar pregnancies in the course of two different marriages, but there has been no evidence of toxoplasmosis in any sizable group of HM cases.

A more startling recent development has been the alleged successful isolation and propagation of a filterable virus presumed to be the cause of HM.⁴⁰ Vesicles from a case of HM "in process of chorionepitheliomatous transformation" were passed through a Seitz filter following centrifugation, and the filtrate inoculated on the chorioallantoic membranes of chicken eggs. On incubation of these eggs, complex and characteristic lesions developed within the chick embryos: the ectoderm was said to show cell proliferation, with formation of inclusion bodies within both nucleus and cytoplasm; the mesoderm was said to show a "sarcomatoid" proliferation of connective

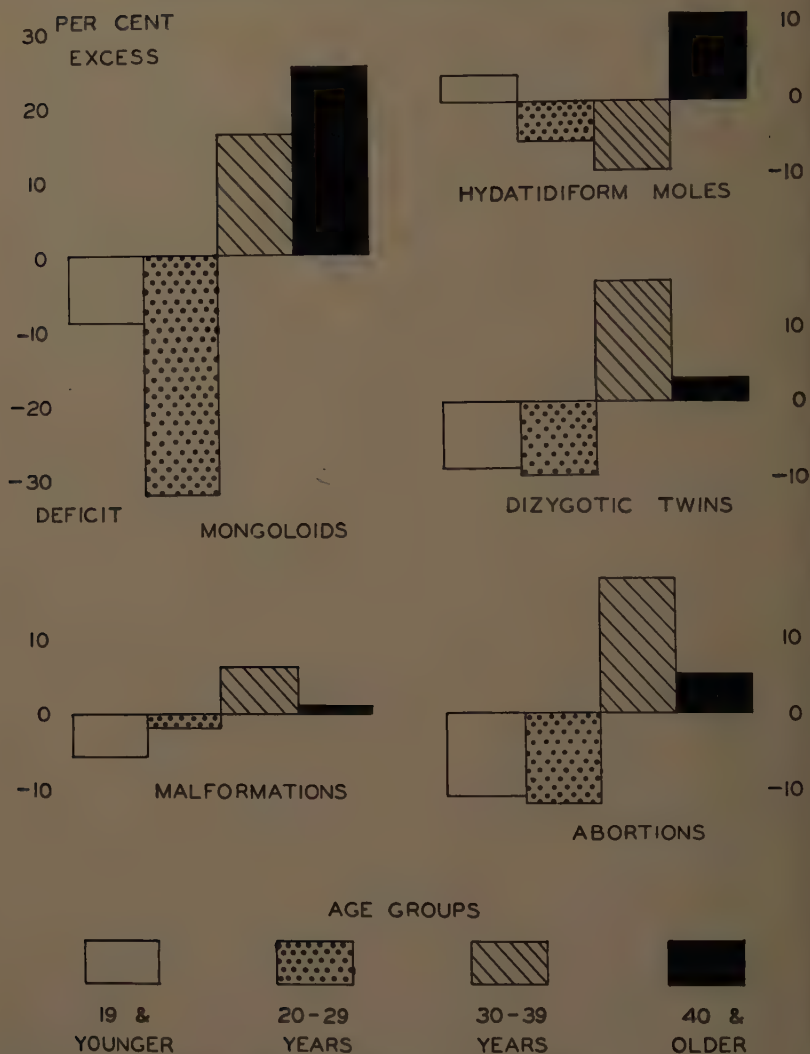


FIGURE 2. Comparative age incidence of HM and various abnormal birth products stated in terms of excess over (or deficit under) the number of all births in the corresponding age groups of the general population. In the ideogram representing HM, for example, the bar for age group 19 years and younger shows a deviation of +3.2 per cent, since 10.2 per cent of all HM cases collected from the literature in this study appeared in this age group while only 7.0 per cent of all births in the general population occurred in this age group.

tissue, also an arborescent proliferation of endothelium; "papillomas" were described in the endoderm and on the amnion. The liver manifested "metastasislike" accumulations of cells within blood vessels, resembling the endothelial proliferations.

All of these lesions were said to reappear on successive passages (numbered

not indicated) from egg to egg. Controls, including eggs inoculated with identical filtrates of vesicles, boiled for ten minutes, and eggs inoculated with filtrate of normal placenta failed to develop the lesions.

In addition, electron microscope photographs of presumed viral bodies ranging from 50 to 100 μ in diameter were produced, using material obtained by subjecting vesicles of the same HM to freeze-drying followed by boiling in distilled water and repeated centrifugation of the supernatant fluid.

In a second series of experiments,⁴¹ the same worker used inocula derived from 4 other cases of HM, including 1 case with "pulmonary metastasis." This time a Chamberland L2 filter was employed, and 3 successive passages egg-to-egg were carried out. In order to eliminate the possible presence of placental hormones in the filtrate, the inocula in one case were centrifuged, and the centrifugate resuspended in fresh fluid and refiltered. Lesions essentially similar to those previously demonstrated are said to have been produced in chicken eggs.

Only one author⁴² seems to have attempted confirmation of the extraordinary studies just reviewed. His results are of equal interest. Seitz filtrates were prepared from suspensions of vesicles in 6 cases of HM and were inoculated in chicken eggs. After incubation lesions were encountered, essentially the same as those reported in the eggs of the first experimenter. Four successive egg-to-egg passages were then performed, using suspensions obtained from membranes in which lesions had appeared. Suitable checks were made to ensure bacteriological sterility. Again lesions were produced, but it was observed that these were increasingly less distinct than those of the first inoculation. It was then concluded that the lesions in the chicken eggs were caused "not so much by a transmissible virus, but rather by some agent present in HM tissue and strongly diminishing in concentration in the course of the serial transfer." The possibility that this agent might be gonadotrophic hormone was considered. Accordingly, a series of control eggs was inoculated with a chorionic gonadotrophin preparation (Pregnyl, Organon), while with another control series the inoculum was a mixture of equine gonadotrophin, luteinizing hormone, and follicle-stimulating hormone (Gestyl, Organon). No lesions appeared in the Pregnyl series, but eggs in the Gestyl series showed lesions quite like those induced by inoculations of HM filtrate, except that they were even more strikingly developed. Furthermore, the intensity of the lesions appeared to vary in accord with the concentration of the Gestyl solution. Control inoculations, carried out with hormone solvent, with water, and with tissue from HM heated for 1 hour at 56° C. or at 100° C., were all negative.

The work of two experimenters, therefore, has shown that an unidentified substance, possibly hormonal and probably *not* a filterable virus, is present both in bacteria-free filtrates of HM and in a commercial preparation of equine gonadotrophin, luteinizing hormone and follicle-stimulating hormone, which is capable of inducing characteristic lesions on inoculation into embryonated hens' eggs.

Unlikely as it may seem for HM to be induced by a virus, the possibility is by no means excluded.

Consideration of other exogenous influences, such as diet or nutritional status, that may at least create a predisposition to the development of HM, may be found in the discussion of inequities of worldwide distribution of HM by other research workers elsewhere in these pages.

The occurrence of HM as one of twin conceptuses has been reported at least twice in the last year^{43, 44} and not infrequently in the past. In the typical instance, the delivery of a normal infant and its placenta is either immediately preceded by, or followed by, expulsion of an apparently anatomically separate HM that is generally considered to represent the remains of a twin conceptus. This interpretation is undoubtedly correct.

There seem to be no recorded examples of twin pregnancies resulting in two Hm, although one would expect concordance as well as discordance in regard to development of HM in at least some of these dichorionic twin pairs. Possibly examples of double HM (simultaneous double molar pregnancy) have escaped detection owing to the difficulties inherent in the fragility of the chorionic sac of an HM. It would be difficult to distinguish between two separate sacs and two parts of a single, broken sac.

The significant point is really that the villi of one twin may be quite normal, while those of a co-twin have developed into HM. While this has been cited elsewhere² as evidence that HM proceeds from a primary ovular defect, rather than from an intrauterine abnormality such as endometritis, the possibility of a combined etiology has not been denied. HM could be induced by the combined action of a chromosomal factor present in the ovum and a maternal constitutional or age influence. A comparable situation probably exists in the causation of Mongoloids, where concordance-discordance studies of twins⁴⁵ have indicated the presence of a genetic factor as well as the better-known age effect.

Since production of HM in sequential pregnancies or in multiple, non-consecutive pregnancies of a given mother has been observed, the possibility must be considered that there exists a lethal gene, inducing early death of the embryo. The age differential here demonstrated may affect the penetrance of such a gene.

Summary

Two new sets of data have been developed, bearing on the genesis of hydatidiform mole (HM):

(1) External measurements of normal and abnormal chorionic villi have been presented graphically to demonstrate continuity of development in (a) a series of young villi showing hydatidiform degeneration, (b) a series of transitional moles, and (c) a series of HM.

(2) Age-incidence statistics of more than 1300 HM reported in various consecutive series from United States and European clinics have been collected from the literature, together with corresponding statistics of appropriate control populations, and presented in both tabular and graphic form to demonstrate that disproportionately more HM are produced by mothers younger than 20 years or older than 39 years.

Reported attempts to isolate a causative filterable virus from HM have

been reviewed, showing that present evidence points to a substance that is nonviral, possibly hormonal, present in bacteria-free filtrates of HM and capable of inducing specific lesions on inoculation into chicken eggs.

While other possible theories cannot be excluded on the basis of evidence at hand, preference is given to the hypothesis that HM is a state of altered growth (malformation) of a conceptus, proceeding from interruption or lack of integration of the villar vasculature, probably caused by a complex of variables in the maternal organism, including age. The possibility that a genetic factor is also involved remains to be explored.

References

1. MALL, F. P. & A. W. MEYER. 1921. Hydatidiform degeneration in uterine pregnancy. Chap. VIII. *In* Studies on Abortuses; a Survey of Pathologic Ova in the Carnegie Embryological Collection. Contrib. Embryol. Carnegie Inst. Wash. **12**(56): 203-231.
2. HERTIG, A. T. & H. W. EDMONDS. 1940. Genesis of hydatidiform mole. *A. M. A. Arch. Pathol.* **30**: 260-291.
3. SCHWALBE, E. 1906. Entstehungszeit der Missbildungen und formale Genese. Kap. VIII, Teil I in: *Die Morphologie der Missbildungen des Menschen und der Tiere.* Gustav Fischer. Jena, Germany.
4. McKAY, D. G., C. C. ROBY, A. T. HERTIG & M. V. RICHARDSON. 1955. Studies on the function of early human trophoblast. II. Preliminary observations on certain chemical constituents of chorionic and early amniotic fluid. *Am. J. Obstet. Gynecol.* **69**: 735-741.
5. McKAY, D. G., C. C. ROBY, A. T. HERTIG & M. V. RICHARDSON. 1955. Studies on the function of early human trophoblast. I. Observations on the chemical composition of the fluid of hydatidiform moles. *Am. J. Obstet. Gynecol.* **69**: 722-734.
6. HERTIG, A. T. 1935. Angiogenesis in the early human chorion and in the primary placenta of the macaque monkey. *Contrib. Embryol. Carnegie Inst. Wash.* **25**(146): 37-82.
7. WISLOCKI, G. B. & E. W. DEMPSEY. 1946. Histochemical age changes in normal and pathological placental villi (hydatidiform mole, eclampsia). *Endocrinology.* **38**: 90-109.
8. ZILLIACUS, H. 1958. Polysaccharide structures in normal chorionic villi, hydatidiform mole and chorionepithelioma, with special reference to the chorionic gonadotrophic hormone. *Gynaecologia.* **135**: 161-172.
9. HERTIG, A. T. & W. H. SHELDON. 1947. Hydatidiform mole; a pathologicoclinical correlation of 200 cases. *Am. J. Obstet. Gynecol.* **53**: 1-36.
- 9a. HERTIG, A. T. & H. MANSELL. 1956. Hydatidiform mole and choriocarcinoma. Part 1 of Fascicle 33, Section IX. *In* Atlas of Tumor Pathology. Armed Forces Inst. Pathol. Washington, D. C.
10. HUNT, W., M. B. DOCKERTY & L. M. RANDALL. 1953. Hydatidiform mole; a clinicopathologic study involving "grading" as a measure of possible malignant change. *Obstet. & Gynecol.* **1**: 593-609.
11. MARCHAND, F. 1895. Über den Bau der Blasenmole. *Z. Geburtshilfe u. Gynäkol.* **32**: 405-472.
12. BOIVIN, M. A. V. G. 1827. Nouvelles recherches sur la nature, l'origine et le traitement de la môle vésiculaire ou grossesse hydatique. Méquignon l'aîné père. Paris, France.
13. DEMOGRAPHIC YEARBOOK. 1948. 1st Issue. Statistical Office of the United Nations Department of Education and Social Affairs. United Nations. Lake Success, N. Y.
14. DEMOGRAPHIC YEARBOOK. 1956. 8th Issue. Statistical Office of the United Nations Department of Education and Social Affairs. United Nations. New York, N. Y.
15. KUCZYNSKI, R. R. 1935. The measurement of population growth. Sidgwick & Jackson, Ltd. London, England.

16. HOLMAN, A. W. & E. H. SCHIRMER. 1947. Results of three five-year studies on the hydatid mole and chorionepithelioma on the Pacific Coast. *Western J. Surg. Obstet. Gynecol.* **55**: 525-537.
17. SHERMAN, J. T. 1935. A study of 78 patients with hydatidiform mole. *Am. J. Surg.* **27**: 237-244.
18. STROUP, P. E. 1956. A study of thirty-eight cases of hydatidiform mole at Pennsylvania Hospital. *Am. J. Obstet. Gynecol.* **72**: 294-303.
19. CABRERA, S. H. 1946. Algunas consideraciones sobre mola hidatiforme. *Bol. soc. chilena obstet. y ginecol.* **11**: 107-111.
20. SOMER, S. 1934. Zur Klinik der Blasenmole. *Med. Klin. (Berlin)* **30**: 932-935.
21. TOLENTINO-ARIAS, R. M. 1946. Contribución al estudio de mola hidatiforme en la maternidad "Julia Molina" de Santiago. Doctoral dissertation. Santo Domingo, Dominican Republic.
22. CHARRIERE, J. 1954. Le pronostic de la mole hydatiforme; etude de 23 observations. Doctoral dissertation. Lyons, France.
23. CANDIDO, R. 1942. Rilievi clinico-statistici su 56 casi di mola vescicolare. *Arch. obstet. e ginecol.* (2nd ser.) **6**: 80-94.
24. KEHRER, F. A. 1894. Über Traubenmolen. *Arch. Gynäkol.* **45**: 478-505.
25. KROEMER, P. 1907. Klinische Beobachtungen über Aetiologie und Therapie des Chorionepithelioms, in besondere über die Behandlung der Blasenmole. *Deut. med. Wochschr.* **31**: 1246-1250; 1294-1297; 1328-1331.
26. GIES, F. 1914. Beiträge zur Klinik der Blasenmole. Doctoral dissertation. Heidelberg, Germany.
27. SCHWARZ, D. 1950. Über die Aetiologie von 86 Fällen Mola hydatidosa. Doctoral dissertation. Munich, Germany.
28. THIERFELDER, H. 1936. Zur Klinik der Blasenmole und des Chorionepithelioms. Doctoral dissertation. Leipzig, Germany.
29. BRIGGS, H. 1912. On the relative size of the uterus in cases of hydatid mole, with illustrative cases and specimens. *J. Obstet. Gynaecol. Brit. Empire.* **21**: 1-16.
30. BREWS, A. 1935. A follow-up survey of the cases of hydatidiform mole and chorionepithelioma treated at the London Hospital since 1912. *Proc. Roy. Soc. Med.* **28**: 1213-1228.
31. REMMELTS, R. 1924. Over mola hydatidosa. *Ned. Tijdschr. Geneesk.* **68**: 2584-2599.
32. VAN DONGEN, J. A. 1924. Mijn abortusgevallen; bijdrage tot de kennis der mola hydatidosa. *Ned. Tijdschr. Geneesk.* **68**: 1824-1845.
33. SMALBRAAK, J. 1957. Trophoblastic growths; a clinical, hormonal and histopathological study of hydatidiform mole and chorionepithelioma. Doctoral dissertation. Utrecht, Netherlands.
34. SUNDE, A. 1920. Chorioepithelioma malignum; kliniske og patologisk anatomiske Studier med Bidrag til Belysning av Blaeremolaens Overgang til Chorioepithelioma. Emil Moestueve. Kristiania, Norway.
35. BLEYER, A. 1938. Role of advanced maternal age in causing mongolism; a study of 2,822 cases. *Am. J. Diseases Children.* **55**: 79-92.
36. MURPHY, D. P. 1954. The birth of congenitally malformed children in relation to maternal age. *Ann. N. Y. Acad. Sci.* **57**(5): 503-506.
37. JAVERT, C. T. 1957. Spontaneous and Habitual Abortion. Blakiston. New York, N. Y.
38. YERUSHALMY, J. & E. S. SHEERAR. 1940. Studies on twins; (I) the relation of order of birth and age of parents to the frequency of like-sexed and unlike-sexed twin deliveries. *Human Biol.* **12**: 95-113.
39. BLEIER, W. 1953. Blasenmole und Toxoplasmosis; Beitrag zu einer infektiös-toxischen Genese und zur Kasuistik rezidivierender Molen. *Geburtshilfe u. Frauenheilk.* **13**: 57-64.
40. DE RUYCK, R. 1951. Isolement d'un virus filtrable dans une môle hydatiforme en voie de transformation chorioépithéliomateuse. *Bull. assoc. franc. étude cancer* **38**: 52-104.
41. DE RUYCK, R. 1951. Mise en évidence du virus choriotope dans quatre cas de môle

hydatiforme, et dans un cas de metastase pulmonaire de chorioépithéliome. Bull. assoc. franc. étude cancer. **38**: 252-268.

42. VAN BOUWDIJK-BASTIAANSE, J. H. H. 1957. Enting van mola hydatidosa en chorion-epithelioma op de vruchtvliezen van het kipeëmbryo. Doctoral dissertation. Leyden, The Netherlands.
43. LOGAN, B. J. 1957. Occurrence of a hydatidiform mole in twin pregnancy; case report. Am. J. Obstet. Gynecol. **73**: 911-913.
44. TAYLOR, J. H. 1957. Twin pregnancy; well-formed hydatidiform mole associated with normal viable child. Brit. Med. J. **5027**: 1103.
45. MACKLIN, M. T. 1950. Monozygous and dizygous twins in study of human heredity; mongolism. Am. J. Obstet. Gynecol. **59**: 359.

Discussion of the Paper

H. G. GRADY (*Seton Hall College of Medicine, Jersey City, N. J.*): H. W. Edmonds has given a clear definition of his conception of hydatidiform mole with which I think most students of the problem would agree. Many, including myself, would agree also to the existence of a form intermediate between classic mole and the focal hydatidiform degeneration of many abortuses. However, I cannot accept the view that in the genesis of true mole the abnormal placenta sequentially undergoes these changes for, in my experience with more than two hundred classic moles, only a few transitional moles have been seen. It seems to me that the evidence is strongly against a gradual transformation.

ELEANOR DELFS (*Johns Hopkins Hospital, Baltimore, Md.*): There is one other factor that may bear on the problem of hydatid degeneration as an intermediate stage on the way to hydatidiform mole. That is the biological activity of such tissue. I have had the opportunity to do chorionic gonadotrophin assays on about one dozen patients with the diagnosis of hydatid degeneration, and none has had a significant amount of gonadotrophin and most of them have had none.

It is true that usually these assays were done one day or two after evacuation, but if the tissue were closely related to mole it should be more active as a gonadotrophin producer than it seems to be.

W. W. PARK: I was very interested to note H. W. Edmonds's remark that there is still some uncertainty as to whether overgrowth of trophoblast is a primary or a secondary feature of hydatidiform mole. The possibility that trophoblast is a primary lesion, if not, indeed, the primary lesion, is worth considering further for, in many respects, current views on the genesis of hydatidiform mole are not completely satisfying.

There are two essential abnormalities in a hydatidiform mole: the hydrops of the villi and the overgrowth of trophoblast. The hydrops is currently ascribed to continued secretory activity of the trophoblast in the absence of a functioning fetal circulation, the primary etiological abnormality thus being death of the fetus. The evidence on which this view is based is essentially:

(1) In spontaneously aborted conceptuses, pathological ova occur more frequently in the younger conceptuses (of about 10 weeks' age) than in the older ones (of about 15 weeks' mean menstrual age), the pathological ova showing hydatidiform change in the villi much more frequently (50 to 70 per cent) than the nonpathological ova (5 to 15 per cent).

(2) Among pathological ova, the more pathological the ovum, the greater is the incidence of hydatidiform change in the villi.

(3) The age of transitional moles is approximately 16 weeks; that of classic hydatidiform moles, approximately 17 weeks. Both contain either very defective or no embryos.

The inference drawn from these observations is essentially that, given a pathological ovum, the earlier the embryo dies (the more defective the embryo, the earlier, presumably, was its death), the longer has been the time available for continued trophoblastic secretory activity in the absence of a functioning fetal circulation and the greater, therefore, the degree of hydrops. Where the ovum is nonpathological, the longer possession of a functioning fetal circulation is considered to have militated against accumulation of fluid within the villi, such accumulation being further impeded by a postulated greater density of the stroma and lessened secretory activity of the trophoblast. Also, the classic hydatidiform mole is regarded as the distal end of a continuum whose proximal end is the aborted conceptus with only an occasional hydatidiform villus (which conveniently may be called a hydropic abortion), the degree of hydrops roughly paralleling the degree of abnormality in the embryo. Transitional and classic hydatidiform moles are regarded as forms of abortion that have, for some as yet undiscovered reason, been retained for an unusually long time.

There are some difficulties in the way of accepting altogether this deficient fetal circulation view. There still remain among pathological ova in reported series of cases approximately 30 to 50 per cent that do not show early hydatidiform change. Thus, fetal death evidently can occur quite frequently without necessarily leading to the accumulation of fluid within the villi. Also, if the process of formation of hydropic villi were a continuum from the almost insignificant hydropic abortion at one end to the classic mole at the other, transitional forms of mole should be at least as common as, if indeed not more common than, the classic form. However, this is not true, either in published series of cases or in the experience of most workers in a general pathological laboratory.

Further difficulties arise when the causation of the trophoblastic hyperplasia is considered. There is as yet no satisfactory explanation for this all-important feature of hydatidiform moles. The usual explanation is that the hyperplasia arises in response to the stretching caused by the hydrops of the villi. It is difficult to recall other instances of epithelial behavior of this kind; in fact, when epithelial hyperplasia and overproduction of fluid coexist, as, for example, in cystic basal cell growths of the epidermis or in ovarian cystadenomas, we are inclined, probably correctly, to ascribe the overproduction of fluid to some primary abnormality in the function of the epithelium, not vice versa.

These difficulties are lessened by invoking a concept of primary trophoblastic abnormality. Let us regard trophoblast as being prone to 2 main types of disturbance: (1) an essentially benign disturbance of such nature that the patient suffers no permanent harm as a result, either a simple functional dysplasia or a benign neoplasia that may also cause disturbance of function, and (2) a malignant neoplastic disturbance that frequently leads

to death of the patient; this is, of course, choriocarcinoma. This basic concept is represented in FIGURE 1. An expansion of the benign progression is shown in FIGURE 2. The abnormality or dysplasia of trophoblast may be such as to do no more than render the trophoblast incapable of sustaining life of the embryo; this is manifested clinically as a simple abortion. Alternatively, it may be of such nature as to cause oversecretion of fluid into the villi, overtaxing of the circulatory arrangements of the embryo, perhaps even causing pressure atrophy and disappearance of vessels within the villi, in consequence usually leading to death of the embryo; this is manifested clinically either as a hydropic abortion or, if more severe, as a hydatidiform

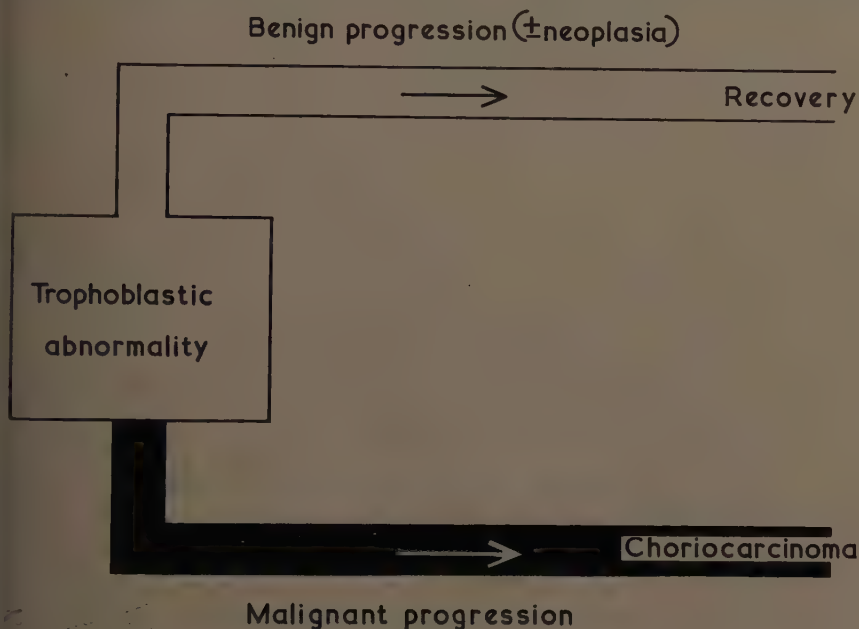


FIGURE 1

mole. There seems no a priori reason why the trophoblastic abnormality that leads in this fashion to the development of a hydatidiform mole should not be regarded as a benign neoplasia of trophoblast. Hydatidiform moles of this kind would be those that are not—and from the essentially benign nature of the causative trophoblastic abnormality never would be—followed by the development of choriocarcinoma.

The malignant progression is shown similarly expanded in FIGURE 3. The essential primary abnormality in each instance is malignant neoplasia of the trophoblast. In the first case the malignant behavior is not manifest until after parturition; this appears clinically as choriocarcinoma following term pregnancy. In the next example the abnormal malignant trophoblast, unable to sustain the needs of the embryo, leads first to abortion and then, by continued growth and after a longer or shorter period of time, to manifest choriocarcinoma; this appears clinically as choriocarcinoma following abor-

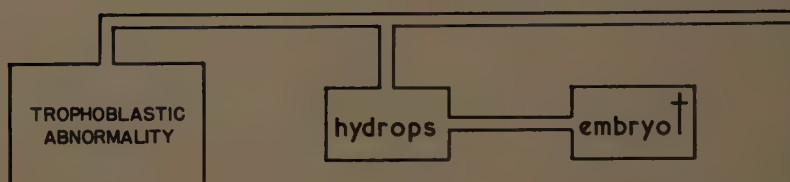
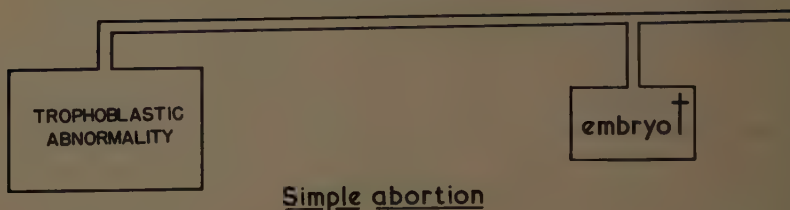
BENIGN LESIONSHydropic abortion or hydatidiform mole

FIGURE 2. Key:†, death of the embryo.

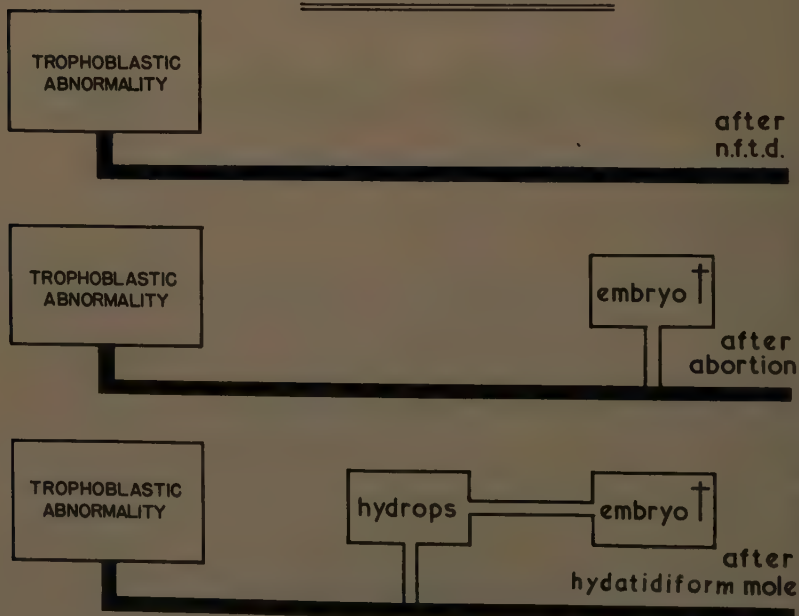
CHORIOCARCINOMA

FIGURE 3. Key: †, death of the embryo; n.f.t.d., normal full term delivery.

tion. Finally, as with the benign progression, the malignant transformation of the trophoblast may so disturb its function as to cause hydrops of the villi and, usually, death of the embryo. This would appear clinically as the familiar hydatidiform mole $\rightarrow$ choriocarcinoma sequence; but the primary abnormality is from the outset malignant neoplasia of the trophoblast (the hydrops of the villi is no more than a by-product, virtually irrelevant except in so far as it acts as a useful stimulus of clinical suspicion).

This concept of primary trophoblastic abnormality, whose various forms or potentials are summarized in FIGURE 4, is as difficult to substantiate by experimental methods as the concept that the hydatidiform mole is caused by primary death of the embryo, but it is a more unifying concept and more

TROPHOBLASTIC "POTENTIALS"

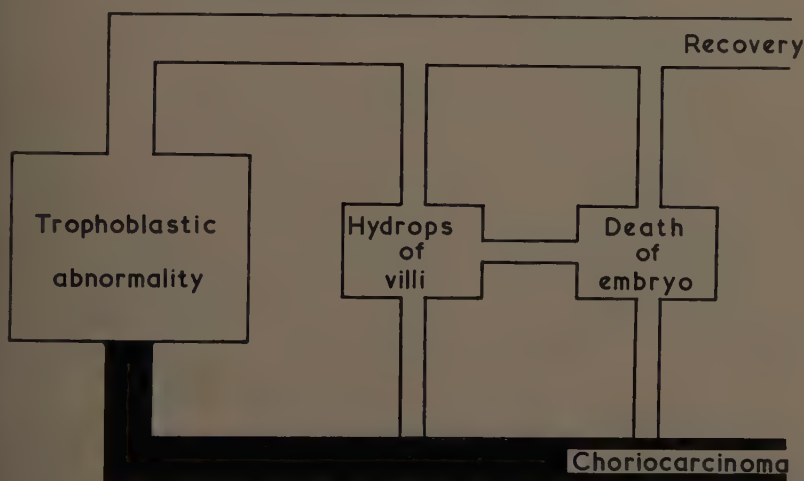


FIGURE 4

economical of hypothesis. By postulating trophoblastic dysplasia of varying degree one can more easily explain the absence of hydrops of the villi in 50 per cent of abnormal embryo conceptuses, the relative rarity of transitional forms of hydatidiform mole, and the curious infrequency of metastases in the so-called chorioadenoma destruens (the trophoblastic abnormality here is presumably such as to endow the trophoblast with great powers of local growth and destruction but with very limited powers of transplantability). Finally, by invoking benign or malignant neoplasia of the trophoblast *ab initio* one is not left in the uncomfortable position of having to believe that, in hydatidiform moles, trophoblast undergoes simple hyperplasia and then malignant hyperplasia as a result merely of stretching.

H. W. EDMONDS: In reply to H. G. Grady's question, I agree that so-called transitional moles are less frequent than might be expected at first. I believe that this is due, in part, to the rigidity of the definition of this middle category, specifying that various parts of the conceptus shall still be identifiable, while the villi show sufficient hydatidiform change for gross recognition.

The 2 other divisions of my classification are augmented by addition of borderline cases. Had a transitional group been defined solely in terms of size or proportions of swollen villi as, for example, "moles with an intermediate or transitional grade of villar swelling," then the three types might have manifested a more nearly equal frequency. One must remember, however, that although my selection of descriptive groups was in part arbitrary, the progressive sequence from early ova showing hydatidiform degeneration (sometimes called hydropic abortions) up to the most mature hydatidiform mole is a natural sequence, not invented but observed.

A second possible explanation of the relative infrequency of transitional moles may lie in a natural tendency for fewer conceptuses to abort, even though abnormal, once the peak of abortion extending from the eighth to the twelfth week of gestation¹ has been passed. The longer a conceptus with hydatidiform alteration of its villi is retained *in utero*, the more likely is it that it has passed through the transitional stage into a mature hydatidiform mole (HM). Indeed, it would seem quite possible that if, as suggested by W. W. Park in his stimulating discussion, we were able to delay the peak of spontaneous abortion from 10 weeks to 18 weeks, we might thereby greatly augment the number of maturing HM, simply because the delay would allow further development from the hydropic-abortion type of conceptus through the transitional stage to HM. By analogy, the fundamental difference between a one-minute egg and a five-minute egg is a mere matter of four additional minutes in boiling water.

In further reply to Park, I must object to the idea that it would be desirable to give our clinical colleagues a prognosis based on histological evidence and couched in terms of a risk of ensuing choriocarcinoma rated 1, 5, or 10 per cent. I do not think that clinicians are able to evaluate such information properly. I feel certain that pathologists at large are not competent to render such a grading, and I have definite doubts that even our "experts" can do the job with much consistency.

In reply to E. Delfs: I am much interested in the fact that she has not been able to demonstrate significant elevation of titer of chorionic gonadotrophin in cases of hydatidiform degeneration of young ova (hydropic abortuses). Since gonadotrophin is produced solely by trophoblast and not by swollen villar stroma, Delfs' finding is additional evidence that hyperplasia of trophoblast is a *late* development in the formal genesis of HM, and not, as Park, among others, has suggested, a primary occurrence.

In answer to another question, I do not know of any evidence suggesting that hydatidiform degeneration of a conceptus is causally related to the occurrence of a manifest viral infection in the mother. I suspect that if a virus is ultimately proved to be a factor in the causal genesis of HM, the virus may be both common and of little recognizable effect in the adult host, similar in these respects to the salivary-gland viruses that have been identified in a variety of mammals.

Reference

1. JAVERT, C. T. 1957. Spontaneous and Habitual Abortion. Blakiston. New York, N. Y.

PROBLEMS IN THE CLASSIFICATION OF HYDATIDIFORM MOLES

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Twelve years ago no one would have known what was meant by the expression classification of moles, for investigations dealing with the subject of grading of moles on the basis of histological examination had not yet been published. In 1947 Hertig and Sheldon were the first to publish an article on this subject. Their paper appeared in the January issue of the *American Journal of Obstetrics and Gynecology* and dealt with 200 cases of mole; in it the authors called attention to the significance of a histological examination of every case of hydatidiform mole, arguing the possibility of predicting with a considerable degree of certainty the serious consequences that may arise after a molar pregnancy.

It seems appropriate to review briefly the article concerned. Hertig and Sheldon gathered their material from 109 medical communities and based their investigation on two criteria: (1) the degree to which the trophoblast resembled or differed from that of normal placental tissue of the first trimester of pregnancy, and (2) the absolute degree to which the trophoblast appeared to be undifferentiated, using the criteria employed in grading any tumor, such as pleomorphism, degree of cellular differentiation, abnormal rate of growth, loss of basement membrane, invasion of stroma, and vascular invasion.

Hertig and Sheldon stressed, that it is imperative to select at least 5, preferably 10, regions for routine study. By doing so they divided the moles of their collection into six groups, using the following designations: I, benign, none to slight hyperplasia of the trophoblast; II, probably benign, slight to moderate hyperplasia; III, possibly benign, hyperplasia with slight anaplasia; IV, possibly malignant, moderate anaplasia with hyperplasia; V, probably malignant, variable hyperplasia and marked anaplasia; and VI, malignant, exuberant trophoblastic growth (variable mitotic activity) with marked anaplasia and often evidence of endometrial invasion. The number of cases in each of these groups was, respectively, 22, 30, 33, 59, 39, and 17; and the number of corresponding malignancies, 0, 2, 4, 10, 20, and 17, respectively, which makes a total of 53 malignant cases out of 200 moles, or a percentage of 26.5. The prognosis of all patients concerned appeared good except for the cases of choriocarcinoma; these, however, are very uncommon and frequently fatal. Two patients of this group died 10 and 16 months, respectively, after passage of the mole, due to choriocarcinoma.

Hertig and Sheldon accepted 4 grades of malignancy that they distinguished as syncytial endometritis, chorionepithelioma *in situ*, chorioadenoma destruens, and choriocarcinoma.

The first group, designated as syncytial endometritis, represented the lowest grade of malignancy. Hertig and Sheldon were in doubt whether this group represents true chorionic malignancy, although they actually included

it as such in their study. Two of the patients, refusing operation, were nevertheless alive and well 7 months and 4 years, respectively, after delivery of the mole.

The second group of supposed malignancy was designated as chorion-epithelioma *in situ*. The diagnosis was based on evidence of local endometrial invasion, exuberant trophoblastic overgrowth with pleomorphism, and loss of basement membrane. Hertig and Sheldon considered these lesions a bona fide manifestation of chorioma, as patients who were not operated upon appeared alive and well years after the passage of the mole; one even became normally pregnant.

With reference to the third group, designated as chorioadenoma destruens, Hertig and Sheldon observed that these tumors rarely give rise to clinical evidence of metastasis. The prognosis is excellent, provided the uterus is removed. Curettage in these cases may be misleading, as the curette cannot reach the tumor. None of the patients has died as a result of malignancy in this group.

The fourth group of chorionic malignancies was designated as choriocarcinoma. Pathologically, this group is invariably distinguished by metastases, commonly to the lungs, brain, vagina, and liver, but they may involve other viscera. The metastases consist of pure chorionic epithelium. They possess a plexiform pattern of undifferentiated trophoblast.

Frequently the uterus gives no clue as to the highly malignant nature of the tumor, and Hertig and Sheldon stated that in 3 of the 5 fatal cases the uterus failed to show any evidence of the primary tumor. Obviously, curettage in these cases cannot contribute to the diagnosis. Death usually occurs within 2 years as a result of metastasis.

It appeared that, with the exception of the 5 cases of choriocarcinoma, all cases had an excellent prognosis, even those of chorioadenoma destruens, provided hysterectomy was performed, for these lesions might have proved lethal from perforation, sepsis, or hemorrhage.

Hertig and Sheldon concluded that the more malignant the mole appeared morphologically, the more likely the patient's life was endangered, thereby accepting a marked but not absolute correlation between the degree of apparent molar malignancy and the tendency of the patient to develop a possibly fatal chorionic lesion. Even after removal of a morphologically malignant mole, it was considered justifiable to adopt a policy of clinically watchful observation until objective evidence of malignancy is obtained, which usually consists of continued postmolar vaginal bleeding, subinvolution of the uterus, and persistent excretion of chorionic hormone. Especially quantitative hormone tests were considered of great value, since these tests usually show not only the presence of living trophoblast, but also whether it is increasing or decreasing in amount.

A second attempt to establish a correlation between the histopathological structure of a mole and the clinical course has been made by Hunt *et al.* (1953). These authors collected and investigated 41 cases of hydatidiform mole from patients treated in the Mayo Clinic, Rochester, Minn., between 1914 and 1950. The histological appearance was the essential subject of

their investigation, and an attempt was made to classify each mole according to the criteria set forth by Hertig and Sheldon, with the hope of discovering other histological features of equal or added prognostic importance. However, as their investigation progressed it became apparent that several of the criteria used by Hertig and Sheldon were of questionable value, namely:

(1) Trophoblastic growth independent of a villus could not be considered a definite sign of malignancy, as it was impossible to determine whether these

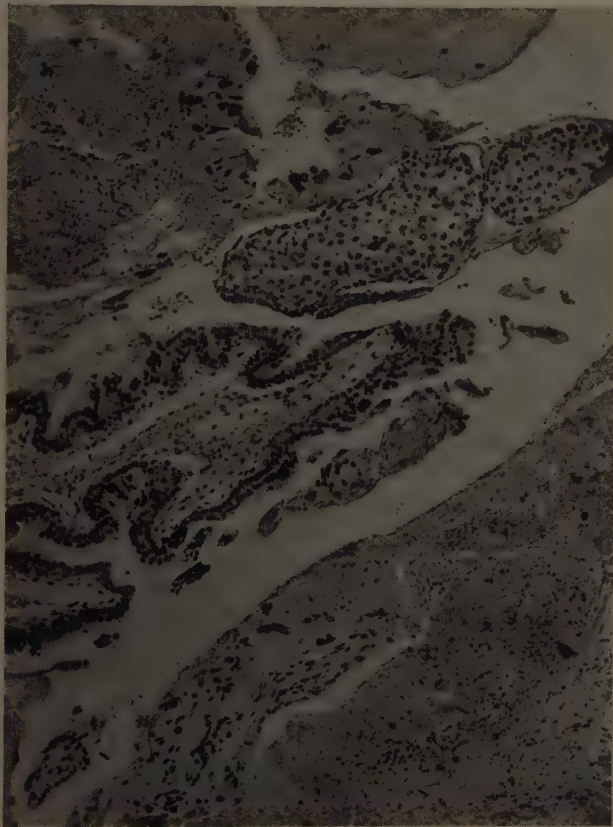


FIGURE 1. Part of a molar vesicle together with a clump of trophoblastic epithelium without stroma found in a case of destructive hydatidiform mole. $\times 80$.

findings represented a section through the tip of a villus or an extravillous growth (FIGURES 1, 2, 3, and 4).

(2) Growth of the trophoblast-resembling tissue culture was likewise most difficult to recognize, as it was impossible to determine whether trophoblastic cells were actually growing in blood or only seemed to do so as a result of the disarrangement of the molar structure, incidental to molar evacuation (FIGURE 5).

(3) Neoplasia and benign hyperplasia in the same microscopic field were

present in most of the moles that exhibited any degree of neoplasia or anaplasia. Hunt *et al.*, in similar cases, attributed the greatest significance to the presence of anaplasia. The mere finding of anaplasia justified the expectation of malignancy.

(4) Villous stromal invasion by adjacent anaplastic trophoblast, considered by Hertig and Sheldon to be evidence of malignancy, was not noted in their series of moles (FIGURE 6).



FIGURE 2. A clump of trophoblastic cells consisting only of cyto- and syncytiotrophoblast, suggesting malignancy in a case of destructive hydatidiform mole. $\times 150$.

Since the above criteria were found to be of doubtful significance, Hunt *et al.* classified their moles on more readily recognizable morphologic characteristics: the degree of anaplasia of the trophoblast and, to a certain degree, the concomitant hyperplasia.

Concerning the practical value of making a detailed histological study of multiple regions of every mole, the results of such analysis were to Hunt *et al.* not conclusive enough to determine definitive treatment, and careful observation of the clinical course of the patient following evacuation of a

mole was considered more important than histological study. As, unfortunately, there is no opportunity at present to enter more extensively into both papers, I must refer to my previous study (Smalbraak, 1957), devoted to the significance of a classification of molar pregnancies.

My study was based on 74 cases of hydatidiform mole, 53 of which approximately fulfilled the requirements of Hertig and Sheldon. The latter cases were classified according to the criteria employed by these authors (FIGURES



FIGURE 3. Tissue space in a uterus, showing deeply invading trophoblastic growth with signs of spontaneous necrosis, found in a case of destructive hydatidiform mole. $\times 150$.

7, 8, 9, 10, 11, and 12), which roughly correspond to their grading. As none of my graded cases developed a malignant chorionepithelioma, the question arises whether the expectation we previously had with regard to the great significance of this system of classification, which was thought to provide an essential guide in the after-treatment of molar patients, was justified.

My study has fully supported the views of Hunt *et al.* with regard to the limitations of the value of grading moles according to histological principles only. In my opinion, the practical value of a classification of moles, designed

to serve as a guide in the treatment of molar patients, has proved disappointing. Besides these objections raised by Hunt *et al.* against Hertig and Sheldon's classification of moles there is, however, another very important point that deserves our fullest attention.

Study of the literature revealed that application of the denominations used by Hertig and Sheldon for those moles belonging to the fourth, fifth,



FIGURE 4. Deeply invading villus of a destructive hydatidiform mole, showing the great vitality of the cytotrophoblast. $\times 150$.

and sixth groups, that is, possibly malignant, probably malignant, and malignant, frequently has induced clinicians to turn to radical surgical measures that in the light of our present knowledge must be considered as much too drastic, depriving the frequently still-young women of the possibility of having children later on. In my opinion this is an illustration of an occurrence not infrequently encountered, namely, that identical expressions are used for completely different phenomena. In my opinion the denomination malignant used by Hertig and Sheldon is in no way so unequivocal as these authors possibly supposed. Obviously, the clinician is

alarmed on being informed by the pathologist that he has to deal with a malignant or with a probably malignant manifestation of some lesion.

If any study is followed by a classification and if terms are used indicating an increasing degree of malignancy, the danger of a misunderstanding in one form or another is easily created, particularly between the pathologist and the clinician. Having ascertained these misunderstandings, it is without

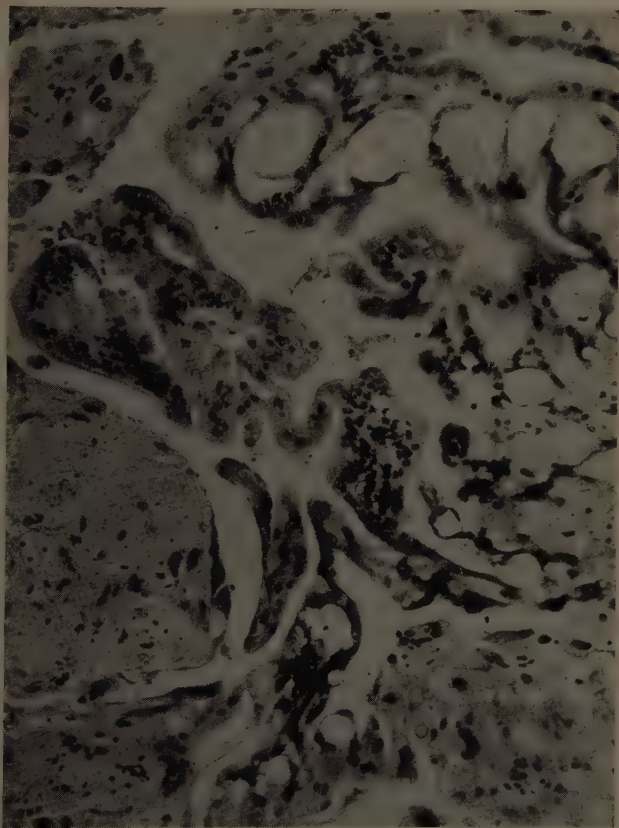


FIGURE 5. Clumps of trophoblastic tissue found in curettings, which might resemble tissue culture. $\times 150$.

any doubt our task to avert the dangers that may follow in their wake. In this connection it must be stressed that the subject of this monograph is an extremely peculiar one, as trophoblastic tissue is not comparable to any other human tissue, for this chorionic tissue, associated with any form of pregnancy, is derived from cells that are not an integral part of the host. Both parents, probably in an equal degree, contribute to the creation of this tissue. Consequently, this tissue is to be considered, to some extent, as a heterogeneous structure. The view point of regarding the tumors derived from these trophoblastic cells as inoculation tumors, as advanced by Schopper

and Pliess (1949) and by Schuster (1952), may help us to understand better some of the unusual properties displayed by these growths. Especially the possibility of a spontaneous regression and even of a complete disappearance seems to be inherent to both tumors.

On the other hand, the normal early trophoblast already shows physiologically typical features of invasiveness and histolytical action, which otherwise are encountered only in cases of real malignancy.

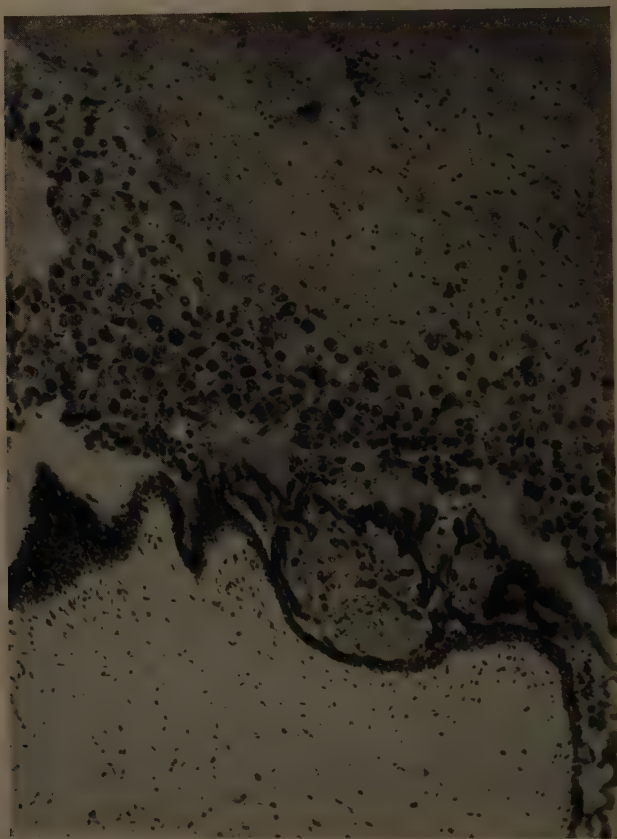


FIGURE 6. Part of a destructive hydatidiform mole showing ingrowth of the trophoblast into its own stroma; found only in cases of high vitality. $\times 150$.

Early trophoblastic cells not merely dissolve the endometrium in which the fertilized ovum is embedded but, occasionally, they also invade the myometrium deeply, opening blood vessels and consequently easily giving rise to dissemination. This may occur even in completely normal pregnancies.

The study of the uterus in cases of normal pregnancy repeatedly has revealed these malignancy-imitating properties of the normal trophoblast. This fact compels us to be extremely reluctant in using terms such as malignant and malignancy while discussing the problems regarding the physio-

logical and pathological products of gestation. Although Hertig and Sheldon insist in their study that there is no absolute clinicopathological correlation, the use of words such as possibly malignant, probably malignant, and malignant in their classification to designate the fourth, fifth, and sixth groups creates an essential danger for all patients who are delivered of a mole graded in these groups, particularly as most young and inexperienced gynecologists, obstetricians, and general practitioners frequently have had neither time nor



FIGURE 7. Molar vesicles showing very slight hyperplasia of the trophoblast, graded in the first group of Hertig and Sheldon. $\times 80$.

opportunity to study thoroughly the peculiar problems regarding these growths. Consequently, the patient who has given birth to a mole belonging to one of these so-called malignant groups is not infrequently subjected to a hysterectomy, a procedure that is only rarely justified.

Further investigation and follow-up of the patients concerned have revealed that even the women who were delivered of a mole with the most malignant appearance, if treated adequately, later on have had normal pregnancies.

Another difficulty inherent in this classification is brought about by the fact that every mole varies in microscopic appearance from portion to portion. Acknowledging this, Hertig and Sheldon stressed the necessity of selecting at least 5, preferably 10, different regions for routine study, suggesting that this arbitrary requirement would make it highly improbable that the most active part of the mole would not be included. Unfortunately,



FIGURE 8. Example of a molar vesicle that showed slight to moderate trophoblastic hyperplasia, considered probably benign. $\times 80$.

however, even this procedure does not guarantee that we have succeeded in obtaining for examination the most active part of the mole.

Frequently the material available for examination is derived from the expelled mole, obviously showing the least active part, whereas the tissues obtained by means of a curettage contain the more active part. Molar villi of the greatest vitality are generally found in the uterine wall, where they are inaccessible to the curette. This does not imply that all patients harboring these moles should be submitted immediately to a hysterectomy. Hor-

monal studies performed in patients submitted to a curettage after expulsion of a mole and revealing a prolonged excretion of chorionic hormone demonstrated the presence of active chorionic cells, sometimes even during a period of several months, without showing any evidence of a developing malignancy. The most plausible explanation in these cases seems to be that the uterine muscle has harbored, temporarily, these clinically benign trophoblastic structures.



FIGURE 9. Example of a mole that showed moderate hyperplasia and slight anaplasia, considered possibly benign (third group of Hertig and Sheldon). $\times 80$.

Considerable difficulties originate in the diverging views regarding the degree of malignancy of penetrating moles. By Hertig and Sheldon they are considered one of the malignant chorionic lesions. In the United States they are frequently designated as chorioadenoma destruens. In my opinion, however, we are dealing only with a hydatidiform mole of high vitality but of limited malignancy, as the tumor always consists of epithelial cells and stroma, thereby exhibiting a state of differentiation, which is always absent in cases of real malignancy. Another feature of the relative benignity is

the fact that the tumors rarely show any tendency to metastasize. In the great majority of cases the growth remains confined to the uterus.

Although the incidence of malignancy in the fifth group of Hertig and Sheldon increased to more than 50 per cent and, in the sixth group, even to 100 per cent, we must consider that these high percentages of malignancy



FIGURE 10. Example of a mole of group IV showing moderate anaplasia with variable hyperplasia of the trophoblast, considered possibly malignant. $\times 80$.

in no way signify a real threat to the life of the patient, as the authors emphasized that the prognosis, paradoxically, for the patients suffering from these malignant lesions was good, with the exception of the 5 patients, of 200, suffering from choriocarcinoma. This unexpected conclusion requires an explanation that, in our opinion, is found merely in the exaggerated use of the designation malignant.

With the exception of the women suffering from choriocarcinoma, none of the 200 patients of Hertig and Sheldon died after their molar pregnancy. In the series of Hunt *et al.* none of the patients developed choriocarcinoma. A similar result was obtained in my series, in which no case of choriocarci-

noma could be ascertained. The only woman who died did not show any evidence of choriocarcinoma; her death was necessarily ascribed to a septic condition following her fourth recognized molar pregnancy, which occurred in her twelfth gestation. All of her other pregnancies, except one, ended in an abortion.

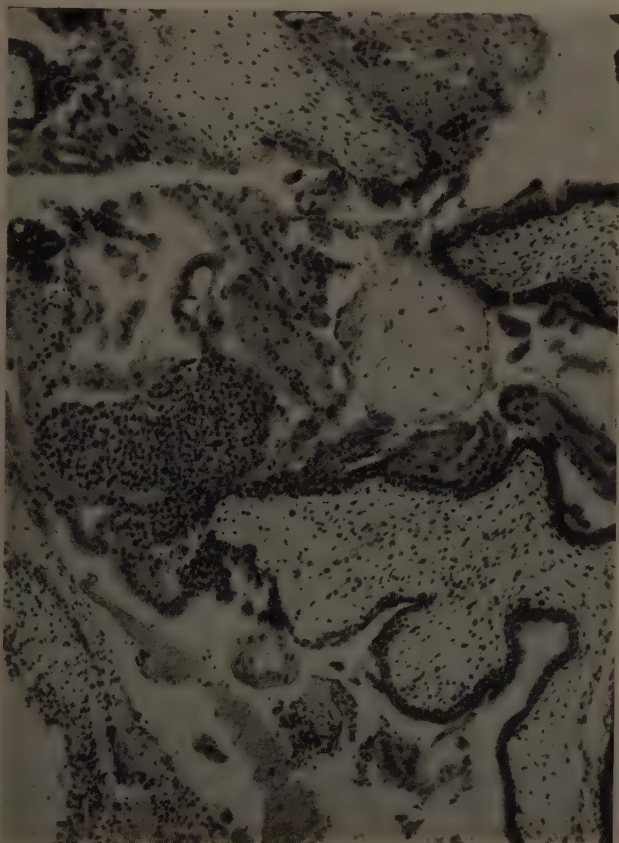


FIGURE 11. Example of a mole showing variable hyperplasia and marked anaplasia of the trophoblast (group V), considered probably malignant. $\times 86$.

Finally, I propose to comment on the terminology regarding the truly malignant manifestations, designated by several terms more extensively reviewed in my previous study (Smalbraak, 1957). As I have tried to make clear there, terms such as deciduoma malignum (Chiari, M. Sanger, Pfeifer), hemorrhagic sarcoma (Guttenplan), epithelioma papillare uteri (H. Meyer), blastoma choriodeciduo cellulare (G. Schmorl) and other such terms do not seem advisable, as they are either terms that do not represent clearly the true character, or are misleading in that they do not represent the real origin and structure of the tumor.

Against the terms trophoblastoma and chorionepithelioma no essential

objections can be advanced, as these designations represent accurately both the real origin and the real structure.

The name choriocarcinoma, so frequently used since J. Ewing and later on by Hertig and Sheldon does not seem appropriate, as these malignant lesions show only some characteristics that may be considered typical for carcinoma. These lesions do possess a tendency to metastasize, but this usually happens

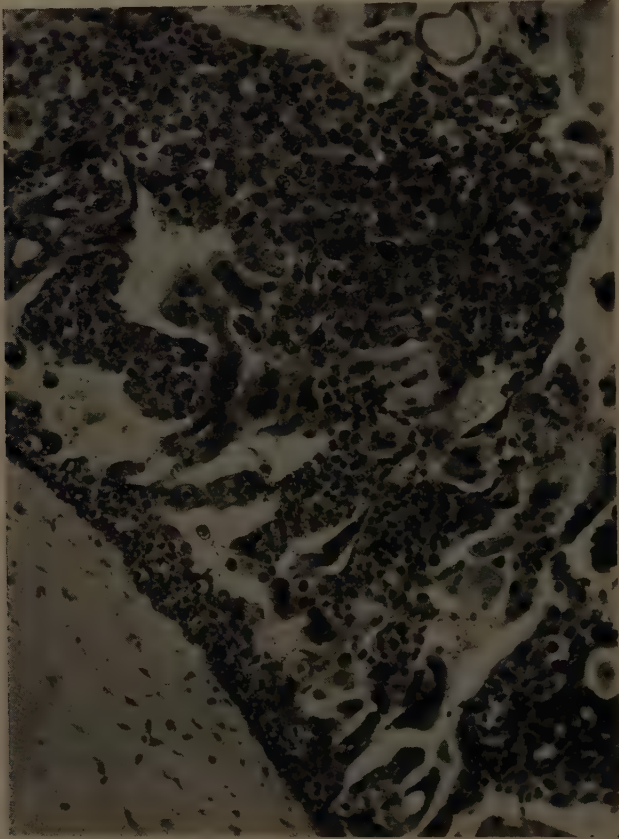


FIGURE 12. Example of a mole of group VI which showed exuberant hyperplasia and marked anaplasia of the trophoblast, considered malignant. $\times 135$.

by way of the blood stream, commonly to the lungs, the brain, the vagina, and the liver.

On the other hand, these tumors show several features that are in no way typical of the really malignant tumor commonly designated as carcinoma. The metastases, like the original tumor, consist only of chorionic epithelium, that is, without any trace of stroma. Consequently, they lack any vascularization of their own. This very lack of stroma should prevent us from considering a malignant chorionepithelioma to be a real carcinoma. Also

these malignant-appearing growths exhibit, in other respects, more vagaries than any other type of common cancer. Even dense shadows on an X-ray photograph of the thorax, suggesting an enormous extension of the malignant tumor, may prove, on follow-up, to have disappeared entirely. Such an occurrence, not uncommon in cases of chorionic malignancy, is hardly imaginable in cases of real cancer.

All these differences must be ascribed to the unique place that these growths occupy in medical pathology. We must deal, in part, with heterogeneous structures displaced in an early previllous stage of ovular development. Although trophoblastic cells in every form of pregnancy may retain their malignant properties, the incidence in which the abnormalities occur seems to be proportional to the degree in which ovular development is disturbed.

This may be the reason why malignant development is encountered so extremely rarely during or after a normal pregnancy. The incidence is already higher in cases of abortion. Frequently abortions are marked by the absence of vascularization in the villi; still, they do not show distinct epithelial hyperplasia. The incidence of chorioneplithelioma is found to be highest in cases of molar pregnancies that are characterized not only by an absence of any functioning vascularization in the villi, but also by an increasing amount of hyperplasia and anaplasia of the trophoblast. The latter characteristic seems to be the more important. These anaplastic cells may give rise more easily to dissemination; detached from the ovular conglomerate, they may retain their peculiar and malignant properties in an autonomous but vulnerable existence.

Therefore, both names—chorioadenoma destruens and choriocarcinoma—do not seem to be felicitous terms for, in the first case, we are not dealing with an adenoma and, in the second case, the name carcinoma does not seem correct because these malignant growths lack many essential characteristics of real carcinomas. Neither term contributes essentially to our knowledge of the real nature of these growths, which are so peculiar in their behavior. Our conception of these growths is not served by the use of designations that are, in fact, misleading and may consequently prove harmful to the patients concerned. That is why, in my study *Trophoblastic Growths* (Smalbraak, 1957) I have rejected the terms chorioadenoma destruens and choriocarcinoma in favor of the older designations destructive hydatidiform mole and chorioneplithelioma. These designations, in my opinion, are less open to misinterpretation and therefore more adequate, as they denote the true origin and the real nature of these growths. It is perhaps even more important that these terms symbolize, to a certain degree, the unique place that these lesions occupy in medical pathology.

References

- HERTIG, A. T. & W. H. SHELDON. 1947. Hydatidiform mole: a pathologico-clinical correlation of 200 cases. *Am. J. Obstet. Gynecol.* **53**: 1-36.
- HUNT, W., M. B. DOCKERTY & L. M. RANDALL. 1953. Hydatidiform mole: a clinico-pathologic study involving "grading" as a measure of possible malignant change. *Obstet. & Gynecol.* **1**: 593-609.

- SCHOPPER, W. & G. PLIESS. 1949. Über Chorionepitheliosis. Ein Beitrag zur Genese, Diagnostik, and Bewertung ektopischer chorionepithelialer Wucherungen. Virchow's Arch. pathol. Anat. u. Physiol. **317**: 347-384.
- SCHUSTER, A. 1952. Genese und Therapie des Chorionepithelioma malignum. Arch. Gynäkol. **181**: 477-499.
- SMALBRAAK, J. 1957. Trophoblastic Growths. Chap. IV. Elsevier. Amsterdam, Holland. D. Van Nostrand. New York, N. Y.
- Idem.* Chap. VI.

Discussion of the Paper

H. G. GRADY: J. Smalbraak's paper summarizes many of the points presented in his previous monograph *Trophoblastic Growths*. His criticisms of Hertig and Sheldon's paper of 1947 are well taken, but in all fairness it must be pointed out that Hertig and Mansell,¹ in their more recent fascicle published in 1956, modified the original position. I can also testify to the difficulties of classifying moles in 6 groups on the basis of histological appearances. A grouping of 3 based on quantity and appearance of covering trophoblast, however, is possible and seems, at least, to give a rough approximation of the risk of subsequent choriocarcinoma.

The criticisms of terminology require comment. I think it is the consensus among United States pathologists that the use of the suffix carcinoma in choriocarcinoma is justified by the view that trophoblast is an epithelium in every sense of the word and that there is no doubt of the malignancy of the lesion. The predilection for metastasis by blood vessels does not seem pertinent, for other carcinomas, for example, thyroidal carcinoma, may do likewise. The strictures against Ewing's term chorioadenoma are sound, and the only justification for retaining it is custom (as we retain the term cystosarcoma phyllodes for a tumor that is only exceptionally malignant and not very often leaf-like).

Reference

1. HERTIG, A. T. & H. MANSELL. 1956. In Tumors of the Female Sex Organs. I. Hydatidiform mole and choriocarcinoma. : 15. Washington, D. C.

H. W. EDMONDS: I am glad that J. Smalbraak discards the term chorioadenoma destruens in favor of destructive mole, as this change serves to emphasize my belief that a mole, that is, a conceptus with hydatidiform change of its villi, is not a true neoplasm. The old term of Ewing should be remembered only out of historic interest.

I further agree with Smalbraak in feeling that overemphasis on morphologic grading of moles is misleading. Form grading is not likely to succeed in a situation where there is but little difference between the grades proposed. While the grading of certain malignant neoplasms has proved a useful prognostic tool in some situations, it must be remembered that hydatidiform mole is not a malignant neoplasm, not even a benign one. If a scheme of prognostic significance for grading moles is to be developed, it may be anticipated that this will be possible only when we become able to evaluate the factor or factors of resistance of the maternal host to trophoblast.

MANAGEMENT OF PATIENTS WITH HYDATIDIFORM MOLE

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Since my assignment is to discuss the treatment of the patient with hydatidiform mole, I shall dwell only briefly on the incidence and diagnosis of this malady as these aspects of the disease are discussed more fully elsewhere in this monograph. However, in connection with these aspects of the disease, a few brief remarks may not be out of order because, after all, diagnosis is a part of therapy.

Acosta-Sisson at the International Federation of Obstetrics and Gynecology, Second World Congress, in Montreal, Canada in June, 1958 stated that the incidence of hydatidiform mole and choriocarcinoma was about 1 in 145 in the Philippines, 1 in 232 in Japan, and 1 in about 3000 in the United States. However, Eastman¹ gives us a figure for the United States of about 1 in 2000. She attributes an eight- or tenfold increased incidence in China to miserably poor nutrition and, possibly, chronic illness such as tuberculosis rather than to any genetic oriental characteristic. She would therefore have us believe that if prevention is good therapy, adequate diet, especially of protein, mineral supplements, and vitamins, reduces the incidence of these trophoblastic abnormalities. If this is correct, I assume that in making a diagnosis of hydatidiform mole we should expect to find it more frequently in individuals of poor economic and therefore poor nutritional status in the United States. I do not know if this is true.

Although the diagnosis of hydatidiform mole is occasionally quite easy because of the finding of characteristic grapes in the vaginal discharge, this convenient diagnostic sign is often missing, and the diagnosis must occasionally be made by inference.

Undoubtedly the most common diagnostic sign is irregular, sometimes fairly profuse, bleeding; however, since this is also a very common diagnostic sign of incomplete abortion, other identifying characteristics must be sought.

As is well known, the mole grows more rapidly in size than the normal embryo and trophoblast so that a uterus that is larger than the period of gestation would indicate must be viewed with suspicion. It is interesting to note, however, that if a mole is not identified by the twentieth to twenty-second week of pregnancy, thereafter the uterus, may, actually be smaller in size than the uterus containing a normal pregnancy because the latter continues to grow and may outstrip its pathological counterpart in size in the next subsequent month. This latter situation rarely occurs, however, because almost always the mole is diagnosed or spontaneously aborted before this time.

Acosta-Sisson has a rather interesting way of diagnosing a mole which I view with a certain amount of apprehension but which, I suppose, is safe in the hands of an expert operator. When, because of bleeding or uterine

size, the possibility of hydatidiform mole arises, she gently inserts a sound through the internal os; if the sound meets the resistance of fetal membranes she then concludes that the conceptus is a normal pregnancy. Since, of course, the hydatidiform mole has no membrane, a sound through the internal os will meet no resistance; if this is the case she inserts a small polyp forceps through the os and confirms her diagnosis by the tissue that is removed.

In that part of the world where hydatidiform mole and choriocarcinoma are as common as they are in the Philippines, this is probably a useful procedure, but it would indeed be unfortunate to rupture membranes inadvertently by this procedure in a normal pregnancy that might otherwise continue to viability. Although other contributors to this monograph discuss the subject, I shall briefly mention here a quantitative test of chorionic gonadotrophin in the differential diagnosis of hydatidiform mole. These trophoblastic abnormalities are positive in extraordinarily high dilutions but, of course, it is a well-known fact that normal trophoblastic tissue produces a large amount of chorionic gonadotrophin also up to about 12 weeks with a usual peak at about 8 or 10. Therefore, at this stage of pregnancy, the quantitative differential is by no means as great, and considerable care must be exercised in quantitatively evaluating this assay. The toad test for chorionic gonadotrophin has been especially sensitive in this respect, however, in my laboratory, and a quantitative assay on a 12- or 24-hour urined specimen has been valuable in this type of differential diagnosis.

Once the diagnosis has been established by any of the above-mentioned techniques, the treatment is twofold. The first aspect of treatment is to empty the uterus efficiently, satisfactorily, and completely; the second is to observe the patient carefully for at least one year in order to identify any malignant sequel to the mole, should it occur.

Evacuation of the uterus sometimes is not an easy procedure. The uterus is not only larger than the period of gestation would indicate, but it is also likely to be even more vascular than the very vascular organ of normal pregnancy. The uterine wall is thin and boggy and does not provide the vaulted protection against perforation by the curette or other instrument that the uterine wall of even a three months' pregnancy provides. Thus, if the cervix is dilated and curettage attempted, the hazards of hemorrhage and perforation are greater than those that occur in, for example, completion D and C for an incomplete abortion, and no evacuation of the uterus should be attempted without ample blood on hand for necessary replacement should the need occur. The use of large blunt instruments for removal of molar tissue is advisable. Frequent palpation of the uterine fundus during curettage is a useful clinical guide.

The hazard of dilatation and curettage is such that trial of oxytocin induction is recommended even though the mole might be identified as early as 8, 10, or 12 weeks. Certainly, repeated attempts at this type of induction should be made in cases where the uterus is greater than the size of a 3-months' pregnancy. Should the first oxytocin induction fail, it is worthwhile making several more attempts before resigning oneself to operative interference. Should the oxytocin induction be successful, however, it must

be remembered that it is most important to follow this procedure with thorough curettage in order to remove any molar remnants that possibly may exist.

Once the uterus has been satisfactorily evacuated, a quantitative chorionic gonadotrophin assay should be carried out once a week for a period of 6 weeks and, even if negative at this point, should be continued about once a month for 1 year. It is not unusual for a chorionic gonadotrophin assay to remain positive for as long as 1 month or 6 weeks, even in the case of a benign mole; even though this test remains positive, it should be in decreasing concentration if the mole is benign. Should a sudden increase in excretion of chorionic gonadotrophin occur, it should, of course, be carefully checked and, if verified, it casts suspicion on the mole and the possibility of malignant degeneration. Under these circumstances, chest X ray should be carried out and, if negative, immediate complete hysterectomy should be done. It is not advisable at this point to carry out another curettage in the hope of removing the suspicious tissue because the tissue may have buried itself deeply within the myometrium and thus be inaccessible to the curette.

There is one point about which the physician and patient should be most inquisitive and that is the possibility of another pregnancy occurring during the time when these assays of chorionic gonadotrophin excretion are being carried out. A sudden increase in chorionic gonadotrophin would naturally occur in the event of a new pregnancy and this possibility, of course, must always be considered.

If the chorionic gonadotrophin assays gradually decrease in concentration and do not rise again within one year, it is reasonable to assume that the patient is in no great danger of any residual molar tissue undergoing malignant degeneration, although cases have been reported of apparent recurrence even after a number of years.

Reports of successful treatment of choriocarcinoma with methotrexate are certainly encouraging. Because this substance does not pass the blood brain barrier, recurrences of the malignancy have been reported in the cerebral area. It does not seem unreasonable to believe, however, that further pharmacological investigation will result in modifications of this substance that will suffuse brain as well as other tissue.

Reference

1. WILLIAMS' OBSTETRICS. 1956. N. J. Eastman, Ed. 11th ed. : 574. Appleton Century-Crofts. New York, N. Y.

Discussion of Paper

WILLIAM B. OBER (*Knickerbocker Hospital, New York, N. Y.*): One of the important clinical features often associated with hydatidiform moles is toxemia. Estimates of its frequency in this context range up to 40 per cent. Using the criterion of hypertension of 140/90 or more with proteinuria, Douglas¹ found it in about 10 per cent of 167 cases of hydatidiform mole. If the criterion was relaxed to include transient elevations of blood pressure or transient proteinuria, the incidence rose to more than 25 per

cent. When present, toxemia may furnish a clue to diagnosis, for toxemia without antecedent hypertensive or renal disease is rare in the second trimester. While the toxemia associated with moles is infrequently as fulminating as classic toxemia, possibly because the molar pregnancy either interrupts itself or is interrupted in short order, it should be managed with the same care and close observation as toxemia of any sort. The toxemia associated with moles is not due entirely to the fact that there is active proliferation of trophoblast or that there is increased trophoblastic mass with extremely high levels of chorionic gonadotropin. Both of these conditions are present in advanced cases of choriocarcinoma, yet hypertension, proteinuria, and fluid retention are not commonly found. While endocrine factors may contribute to the picture of toxemia, they are insufficient, in and of themselves, as causes.

Systematic follow-up of all patients with hydatidiform mole is essential for the prompt recognition of complications. Douglas¹ has outlined such a plan most succinctly:

"(1) During the immediate period following the passage of a mole: Pelvic examination before discharge from the hospital. Chest X ray. Quantitative biologic test for chorionic gonadotropin.

"(2) Return visits: Weekly visits until the biologic test has reverted to negative and pelvic findings have returned to normal. Monthly visits thereafter for one year.

"(3) Repeat curettage: Indicated after one month if pelvic findings have not returned to normal and the biologic test remains positive. Indicated sooner than one month if findings suggest retained tissue.

"(4) Quantitative biologic tests for chorionic gonadotropin: Should be performed according to the return visit schedule, or more frequently if continued function of trophoblast is evident.

"(5) Chest X rays: Monthly during the first three months, and several times during the ensuing year. Should be carried out immediately upon the occurrence of pulmonary symptoms, or the finding of a positive biologic test."

The practical value of this program is self-evident. The emphasis on quantitative chorionic gonadotropin tests is significant. The day has passed when a qualitative test is sufficient. The patient must be cautioned to avoid another pregnancy during the year of observation in order to avert a mistaken diagnosis of choriocarcinoma because of a rising CG titer.

Reference

1. DOUGLAS, G. W. 1957. The diagnosis and management of hydatidiform mole. Surg. Clin. North Am. : 379-392.

CHORIONIC GONADOTROPHIN DETERMINATIONS IN PATIENTS WITH HYDATIDIFORM MOLE AND CHORIOCARCINOMA

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Chorionic gonadotrophin production is a characteristic of trophoblast in all its forms from the normal pregnancy through the benign hydatidiform mole and the malignant choriocarcinoma.

In normal pregnancy there is a dominance of cytotrophoblast during the period of active placentation. It is at this time that the peak gonadotrophin production occurs, correlating closely with the maximum extent of cytotrophoblast. After the third month both decrease sharply. It is of interest that other primates have the same correlation; for example, the rhesus monkey has a very short gonadotrophin output between 14 and 30 days, mirroring a short span of cytotrophoblastic dominance in the placenta. This cytotrophoblastic growth phase is normally limited, although by what factors is not clear. In hydatidiform mole the limitation does not occur and there is an overgrowth of cytotrophoblast and a concurrent overproduction of chorionic gonadotrophin. It may be stressed that chorionic gonadotrophin seems to be more than a simple quantitative measure of amount of trophoblast; it also seems to be a qualitative expression of a phase of territorial aggression by the trophoblast.

If chorionic gonadotrophin is assayed accurately and correlated with clinical events, it has a twofold function: first, as a method of study of trophoblastic growth activity and, second, as a guide to clinical management. The present discussion will be concerned chiefly with the first, chorionic gonadotrophin as an indicator of growth. Patients with trophoblast tumors have been followed throughout their entire courses and serum chorionic gonadotrophin correlated with events in the natural history of the growths. The relationship has proved to be generally consistent and meaningful.

Method

Quantitation can be made only if a carefully standardized assay is used. Details of the assay method have been published elsewhere,^{1, 2} but it should be noted that all assays have been on blood serum, thus eliminating the difficulties and inaccuracies of urinary assays. The immature 21-day-old rat has been used as test animal, with uterine weight as the end point standardized in international units (I.U.) of chorionic gonadotrophin.

Material and Results

In studying chorionic gonadotrophin production in trophoblastic tumors, two main areas of interest are the differential diagnosis from other benign pregnancy conditions and the period of regression, latency, or transformation to malignancy.

Some benign conditions that may cause clinical diagnostic problems are shown in FIGURE 1. The background chart shows the range of chorionic gonadotrophin in normal pregnancy, with its early peak.³ Any diagnostic problem may be projected against this background for interpretation. A multiple gestation with its large placenta shows a shift to the right of the normal curve, but it follows a normal pattern with a decrease of gonadotrophin as the pregnancy advances. A hydatidiform mole departs from the normal curve and stays high or increases. Another mole is far removed

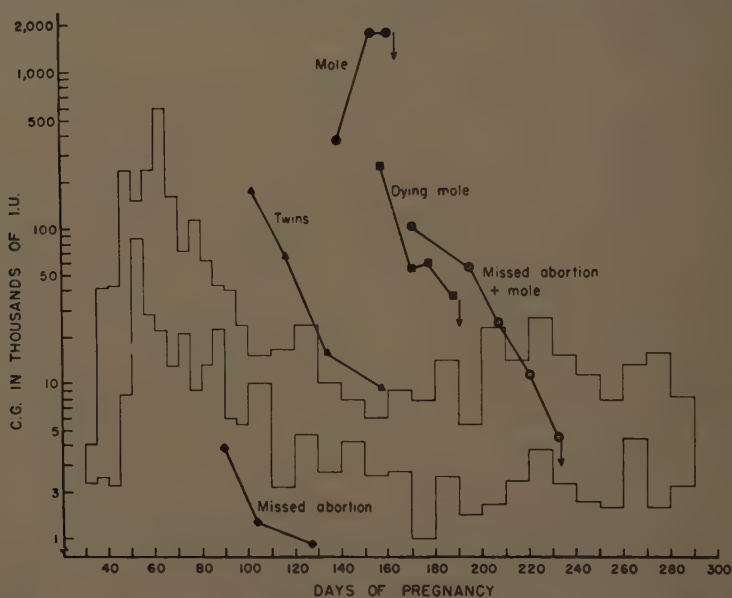


FIGURE 1. Some differential diagnostic problems. The background chart shows the range of chorionic gonadotrophin in normal pregnancy with its early peak. Chorionic gonadotrophin is in thousands of international units. Each curve is labeled with its final diagnosis. See text.

from the normal range, but gonadotrophin decreases as the trophoblast dies *in utero*. The last curve shows an abnormally high gonadotrophin that accompanied a uterus with a fetal skeleton but absent fetal heart. The uterus was evacuated of a missed abortion containing a five-month fetus and a dying mole. In each instance the state of trophoblastic growth was mirrored by the gonadotrophin titer.

The hydatidiform mole with its great tissue overgrowth is already a neoplasm. After its evacuation, remnant cells may die, benign growth may continue or extend, or malignant transformation may take place. Gonadotrophin decreases with regression and increases with resumption of new growth. It does not differentiate between benign and malignant growth, but it does indicate the vigor of growth activity.

One hundred and twenty-nine patients with hydatidiform mole have been studied throughout their courses. Serum chorionic gonadotrophin assays:

were made every 1 to 3 weeks until negative, then monthly for a year. Patients developing invasive moles or choriocarcinoma were followed 5 to 12 years or to death.² The individual growth behavior of each tumor was studied, but all cannot be presented in detail.

TABLE 1 is a summary of the data, a form of actuarial table for moles in general. It is derived from a long study of 119 patients who had moles

TABLE 1
PERSISTENCE OF GONADOTROPHIN AFTER MOLE: 119 CASES

Days after evacuation.....	10	30	60	Assays negative later	Hysterectomy later
Cases with positive assay....	93*	44	26 →	15	11
Percentage of cases.....	78.1*	37.0	21.8	12.6	9.2

* Only one case negative in first 10 days; 25 of 119 cases not seen until later and were negative when first seen.

evacuated but retained their uteri. It shows how long these molar remnants lived. Sixty days after evacuation of the mole there was still evidence of surviving trophoblast in 26 cases, or 21.8 per cent. Fifteen of these gradually regressed and became negative by assay between 70 and 250 days. All of these patients have remained well. The other eleven patients showed persisting or rising assays, in some instances associated with clinical irregularities that eventually led to hysterectomy.

TABLE 2 is the summation of the final outcome of all of the trophoblastic tumors and their hosts. Of 11 patients who came to hysterectomy during

TABLE 2
OUTCOME AFTER MOLE

Evacuation of mole		Initial hysterectomy	Total cases
No. patients.....	119	10	129
Well with uterus.....	108		
Eventual hysterectomy.....	11		
Invasive mole.....	6 (1 death)		6
Choriocarcinoma.....	5 (1 death)	1	6 (4.7%)
Deaths.....	2	1	3 (2.3%)

the follow-up period, 6 had a final classification of invasive mole. Two of these had falling gonadotrophin titers when hysterectomy was done; sections showed dying trophoblast, suggesting that these were regressing spontaneously. One of the invasive moles metastasized to distant sites and finally caused death (see Case M. R. 62 13 59). The other 5 patients were classified as having choriocarcinoma, and 1 of these died. The surviving patients of this complicated group have all been followed for from 5 to 12 years. TABLE 2 also contains an additional 10 cases that had initial hysterectomy as primary treatment, thus eliminating the local site of trophoblast; in



FIGURE 2. Case J. M. Secondary rise of gonadotrophin after mole with spontaneous regression, a rare occurrence.

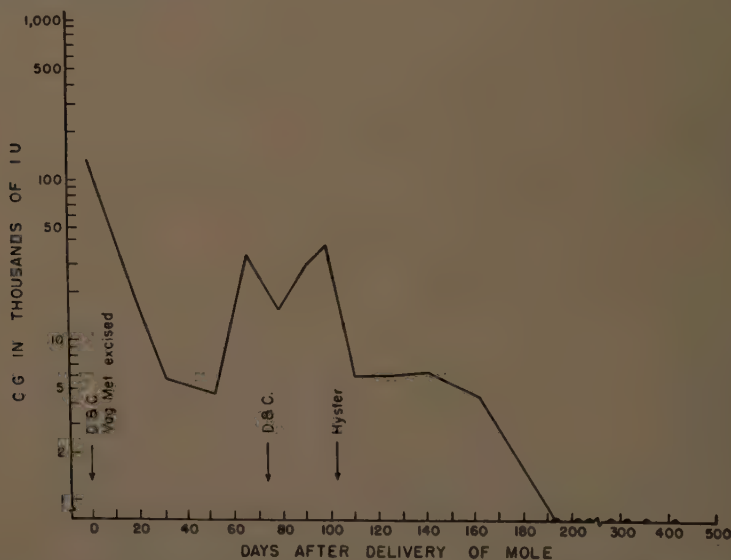


FIGURE 3. Case J. H. 63 16 99. Very early stage of malignant transformation after mole.

spite of this, 1 case had metastases with malignant transformation that proved fatal (Case B. T. 54 73 35).

The life histories of all these tumors are instructive, but only a few can be presented as illustrations of the way in which chorionic gonadotrophin mirrors growth activity.

FIGURE 2 shows spontaneous regression after a period of new growth following evacuation of a mole. Without any therapy this gonadotrophin curve reversed after a few weeks, became negative, and the patient remained

well. Such a course is very rare after a secondary rise and temporizing is not recommended in such a situation. However, occasional bizarre behavior makes prognosis difficult in these tumors.

FIGURE 3 (Case J. H. 63 16 99) shows, from the cellular standpoint, the gonadotrophin output of one of the earliest malignant transformations. This patient had a small vaginal metastasis (with villi) that was excised when the intrauterine mole was evacuated. Subsequent bleeding occurred, curettage yielded no trophoblast, and hysterectomy was done because of rising gonadotrophin titer. The endometrium was normal, but deep in the uterine muscle were a few tiny areas of new growth. FIGURE 4 shows a

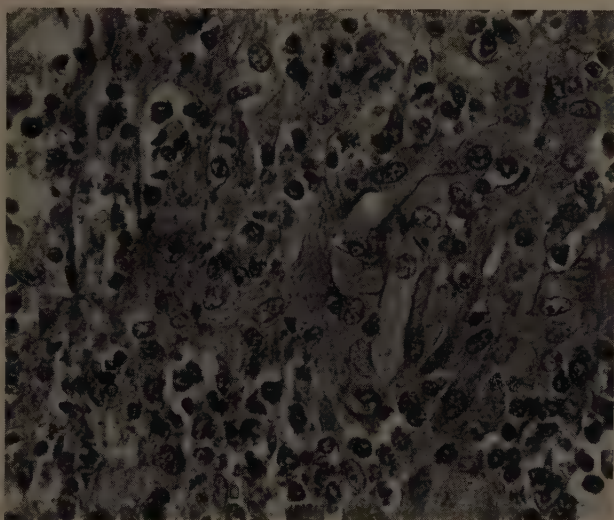


FIGURE 4. Case J. H. 63 16 99. Myometrium with small clusters of malignant trophoblast cells. There are no villous remnants.

small cluster of cells; elsewhere one finds single cells or 2 to 3 very active cytotrophoblast cells creeping between muscle fibers near areas of blood clot. There are no villous remnants. I am not sure that this should be called choriocarcinoma, but it is biologically very potent. One cannot escape the fact that there was a low plateau of gonadotrophin for 2 months after operation in this patient; this plateau then disappeared. This suggests a locus (outside of the uterus) that subsequently died. All examinations have been negative, as are assays in the second year of follow-up. Further observation of this patient is necessary for final evaluation (this case is not included in the 5 to 12 year follow-up reported above).

Case J. R. 59 95 39, shown in FIGURE 5, demonstrates a silent and sudden transformation of growth from indolent to very active type. A benign mole of 3-months' duration was completed by curettage, and the following 2 gonadotrophin assays were very low. The patient had what she considered a normal menstrual period and skipped her assay. When she was returned

for assay somewhat later she had a rising titer and amenorrhea, and the possibility of a new pregnancy was considered. Another episode of bleeding rendered this unlikely, and curettage was repeated. This showed syncytial endometritis with a few clumps of syncytium and small collections of cytotrophoblast without anaplasia. Gonadotrophin was very low after curettage, then maintained a plateau at 5000 I.U. for a month. It then began a steep rise that carried it to 380,000 I.U. in 3 weeks. Hysterectomy was done, the uterus contained a single area of tumor 1.5 cm. in diameter that was typical of choriocarcinoma (FIGURE 6). The patient is well, with negative assays at $6\frac{1}{2}$ years.

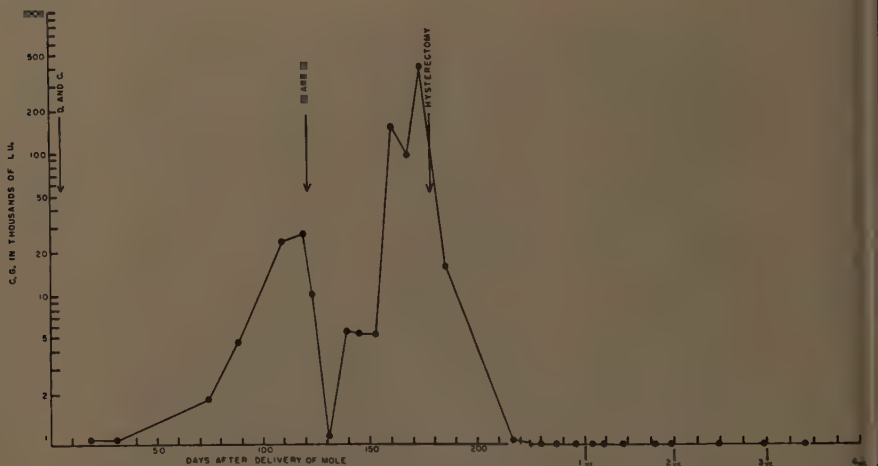


FIGURE 5. Case J. R. 59 95 39. Sudden transformation from indolent to very active growth, suggesting qualitative alteration of cells. No clinical signs at this time. Reproduced by permission from *Obstetrics and Gynecology*.²

The data indicate that trophoblast maintained itself for 5 months after a mole, when it quite suddenly transformed to a malignant growth phase accompanied by a rapid rise in gonadotrophin. The high titer compared with the small tumor mass suggests a qualitative change in cell function. There was no obvious preponderance of cytotrophoblast over syncytial cells in this tumor. Without bioassay, diagnosis would have been delayed, as there were no clinical indications for hysterectomy up to the time of operation.

Vagaries of growth and regression, as well as responses to various agents, are illustrated in the long life histories of the following 2 tumors, 1 a metastasizing mole and the other a choriocarcinoma developing after a benign mole.

The progress of Case M. R. 62 13 59 is charted in FIGURE 7. This patient was not seen in the Johns Hopkins Hospital until the last few months, so that there are some gaps in her assays. A large benign mole was evacuated, followed by a fall in gonadotrophin titer. No further assays were received until after a bleeding episode and curettage when gonadotrophin was 2640 I.U. X ray revealed a single nodule in the lung. Partial lobectomy and

hysterectomy were done. The lung lesion showed a benign mole with villi (FIGURE 8a). The uterus showed scattered penetration into the muscle with villi and small sheets of cytotrophoblast. Gonadotrophin fell to just under 1000 I.U., but rose again as the patient developed paralysis of the legs.

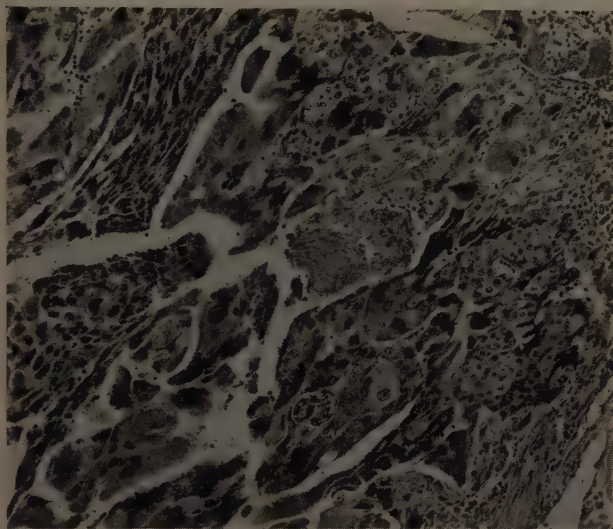


FIGURE 6. Case J. R. 59 95 39. Small area of choriocarcinoma in uterine wall. Patient is well 6½ years later, gonadotrophin negative. Reproduced by permission of *Obstetrics and Gynecology*.²

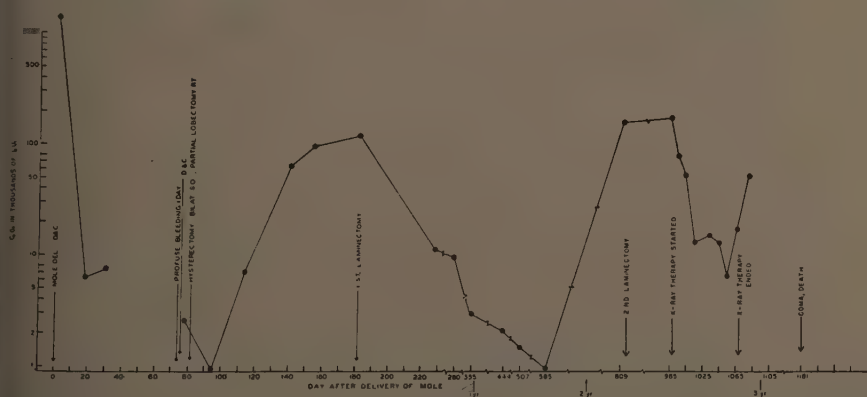


FIGURE 7. Case M. R. 62 13 59. Gonadotrophin production in the long life history of a benign mole metastasizing to distant sites. Twice the assay fell below 1,000 I.U., but was never negative. Reproduced by permission from *Obstetrics and Gynecology*.²

Laminectomy was done, with partial evacuation of extradural metastases of benign mole (FIGURE 8b). Gonadotrophin again decreased to a low of 620 I.U. and neurological signs disappeared. The patient likewise disappeared, returning to her neurosurgeon only when paralysis recurred

7 months later. Gonadotrophin was high and a second laminectomy was done, but hemorrhage made removal of the tumor impossible. X-ray therapy to the lumbar spine produced marked reduction of the gonadotrophin titer and regression in the paralysis in the legs. Near the end of the X-ray

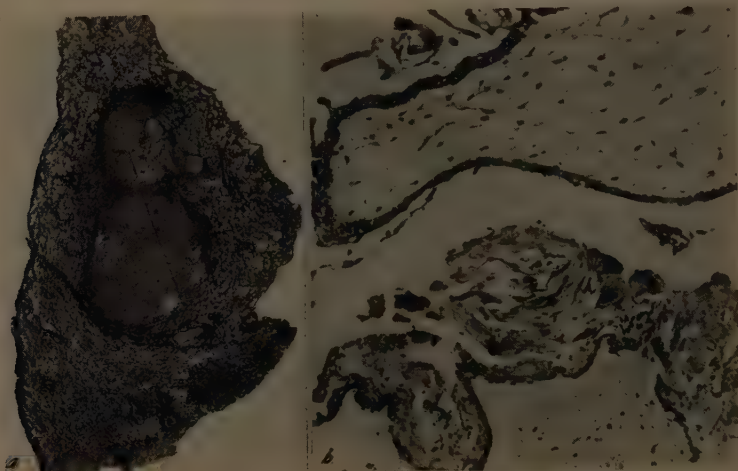


FIGURE 8. Case M. R. 62 13 59. Metastases to lung (a) and extradural region (b) of spine. Both show villi and characteristics of benign mole. Reproduced by permission from *Obstetrics and Gynecology*.²

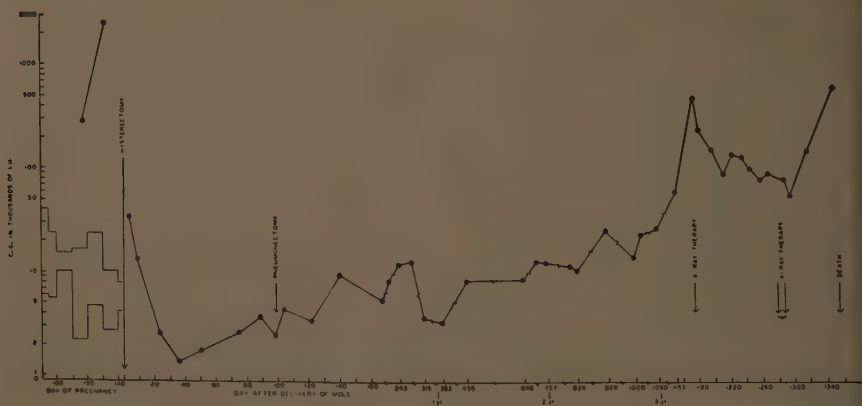


FIGURE 9. Case B. T. 54 73 35. Long survival and slow progression after diagnosis of choriocarcinoma. Reproduced by permission from *Obstetrics and Gynecology*.²

therapy, gonadotrophin began to rise as growth resumed, and paralysis recurred slowly. The patient went into coma suddenly and died 5 months after radiation and 3 years 4 months after appearance of the mole.

This trophoblast certainly maintained a histologically benign pattern in distant metastatic sites for a long time. Twice it regressed spontaneously almost to the vanishing point, only to be reactivated. It responded tem-

porarily to X ray, but was not eliminated. Unfortunately autopsy was not obtained, so it is not known whether this trophoblast ever assumed the characteristics of choriocarcinoma.

Case B. T. 54 73 35 demonstrates a protracted span of a choriocarcinoma (FIGURE 9). The original diagnosis of mole was made by a gonadotrophin assay that showed a rapid rise far out of the range of normal pregnancy. Because of the patient's multiparity, large mole, and toxemia, hysterectomy was carried out as the initial treatment. Gonadotrophin decreased after operation, but persisted at low level for 3 months, when a discrete shadow not present previously appeared in the chest X ray. Pneumonectomy was done on the slender hope of a solitary metastasis. The visualized mass

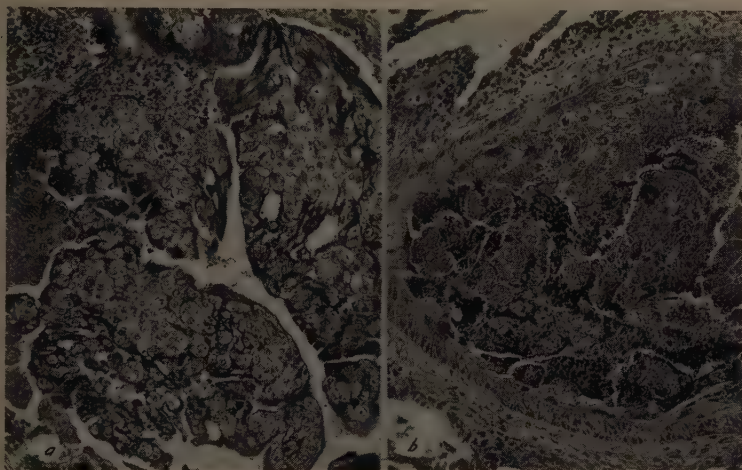


FIGURE 10. Case B. T. 54 73 35. Chorionepithelioma from (a) first lung (pneumonectomy) and (b) from second lung (autopsy) 3 years and 5 months later. The latter shows radiation necrosis. Reproduced by permission from *Obstetrics and Gynecology*.²

proved to be a sarcoid nodule, but a small area in the periphery not seen by X ray proved to be choriocarcinoma (FIGURE 10a). For the next 3 years the patient followed a rather uneventful clinical course, although the gonadotrophin was always positive in the range of 10,000 to 2500 I.U. Attempts to suppress trophoblastic growth by intensive administration of estrogen, progesterone, and testosterone were explored in separate trials. None of these produced any significant change in gonadotrophin level.

After 3 years of quiescence, activation of growth occurred with sudden elevation of gonadotrophin, hemoptysis, and appearance of a 4-cm. lung lesion (not present 3 weeks earlier). X-ray therapy caused disappearance of the lung shadow and a decrease in the gonadotrophin for a time. Gonadotrophin rose moderately before death, which occurred 3 years 5 months after pneumonectomy. Autopsy showed choriocarcinoma scattered in the lung, mostly as strands in small blood vessels (FIGURE 10b). Some areas appeared active, but many were necrotic, probably due to radiation; there

was widespread thrombosis and fibrosis. The heart showed enlargement, especially of the right ventricle, and immediate cause of death seemed to be cor pulmonale secondary to the pulmonary destruction.

The most interesting facet of this case is the survival of the patient for more than 3 years after proved diagnosis of choriocarcinoma. Long latent periods between mole and development of choriocarcinoma have been reported many times, but once malignant change has occurred the course is usually rapidly progressive.

A few examples of trophoblastic tumors* arising from nonmolar pregnancies illustrate the general validity of chorionic gonadotrophin production as an indicator of growth status.

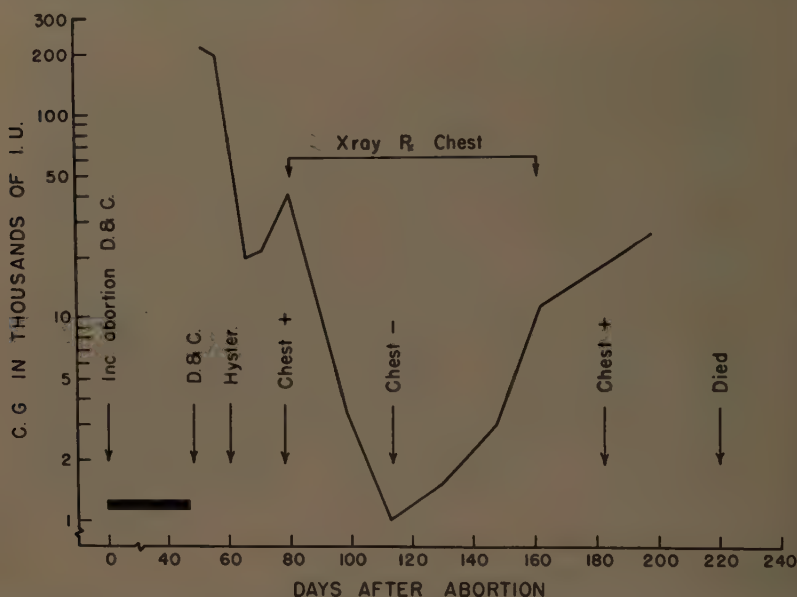


FIGURE 11. Case V. C.—WH 4259. Choriocarcinoma after simple abortion. Lung metastases responded temporarily to X ray, but then recurred.

The history of Case V. C., WH 4259, is charted in FIGURE 11. This patient had curettage for an incomplete abortion. There was no fetus and no hydatidiform change. Bleeding persisted and repeat curettage showed trophoblastic growth. Gonadotrophin assay was found to be high. After hysterectomy choriocarcinoma perforating the uterine wall was found (FIGURE 12). Chest X ray was negative before hysterectomy, but shortly afterward showed characteristic shadows. Therapeutic X ray was given with dramatic response for a time, the gonadotrophin falling to low levels and the chest X ray clearing. Gonadotrophin then began to rise; subsequently metastases reappeared in the chest and the patient succumbed.

* These cases are not included in TABLES 1 and 2 as these patients did not have hydatidiform moles as their precursor pregnancies.

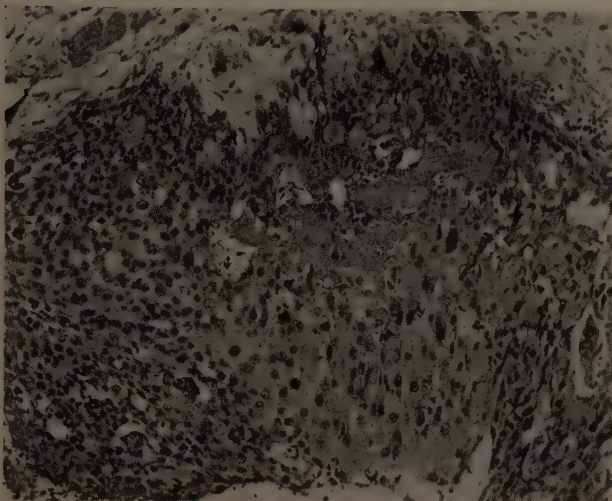


FIGURE 12. Case V. C.—WH 4259. Section from choriocarcinoma perforating uterine wall. Patient succumbed to metastases.

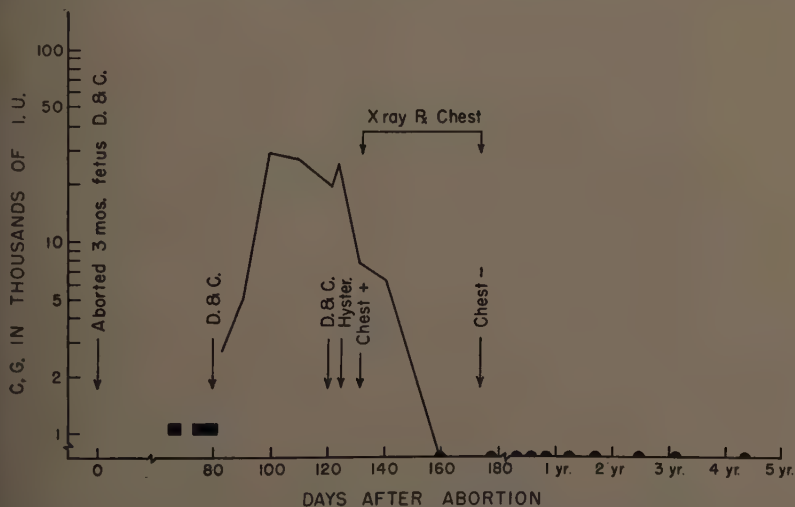


FIGURE 13. Case A. R.—CR 126. Choriocarcinoma after abortion. Lung metastases responded to X ray. Patient well at 5 years; gonadotrophin negative.

The gonadotrophin production indicated the exacerbation well ahead of X-ray or clinical evidences.

Case A. R., CR 126, had an early course (FIGURE 13) almost identical with the preceding case. Abortion of a 3-month fetus occurred and was completed by curettage. Bleeding recurred and curettage was repeated. Gonadotrophin rose moderately and another curettage showed active trophoblast. Hysterectomy was done and choriocarcinoma was found penetrating the

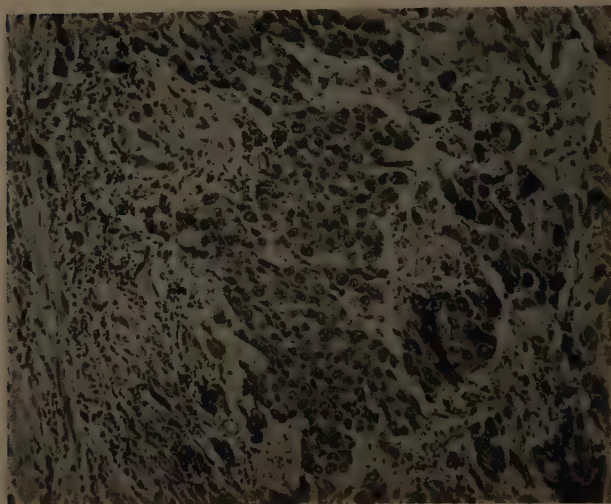


FIGURE 14. Case A. R.—CR 126. Section of choriocarcinoma in uterus. Patient survived. Note the cytological similarity to FIGURE 12 from a fatal case.

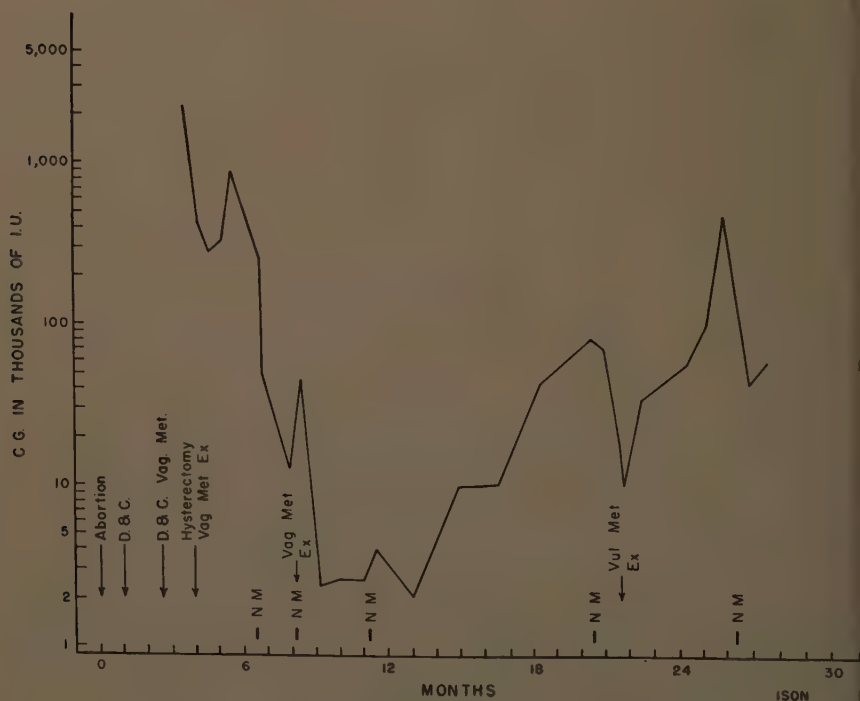


FIGURE 15. Case J. I. 13 24 60. Choriocarcinoma after abortion. Gonadotrophin response to therapeutic procedures.

wall (FIGURE 14). One week later the chest X ray, negative before operation, showed metastases. X-ray therapy was given and gonadotrophin fell rapidly to zero. Chest films were subsequently negative, as were all later gonadotrophin assays. The patient was well at 5 years.

The sequence of events and time relationships in these two tumors was quite similar. The microscopic appearance seems practically identical (FIGURES 12 and 14), yet the growth potential and the sensitivity to radiation were quite different, as were the fates of the patients. The gonadotrophin production bore out this difference.

Case J. I. 13 24 60 had an abortion at 4 months of pregnancy, followed by curettage. There was said to be no evidence of mole. Three months later

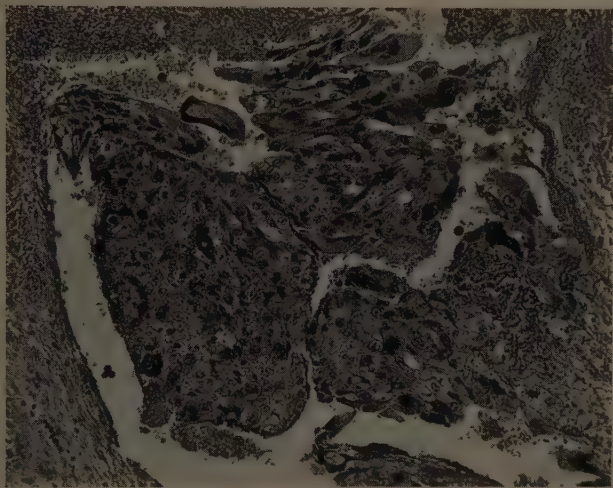


FIGURE 16. Case J. I. 13 24 60. Choriocarcinoma from serosal uterine surface.

curettage was done because of bleeding, and choriocarcinoma was found with a vaginal metastasis already present. The patient was referred for study at this time and had a gonadotrophin over two million U. (FIGURE 15). To control bleeding, hysterectomy and excision of vaginal metastasis was done (FIGURE 16). Gonadotrophin dropped markedly, but then began to rise as a new vaginal metastasis grew rapidly. Nitrogen mustard was given resulting in a dramatic drop in gonadotrophin. The vaginal mass shrank about one half, but was excised later to observe the effect of therapy on the cells (FIGURE 17). There was widespread necrosis, but not complete destruction. Gonadotrophin was very low after further nitrogen mustard. The patient was clinically well; since the white blood count remained low, further therapy was deferred. Gonadotrophin titer rose gradually and a deep vulvar metastasis was found. Nitrogen mustard again had an effect and the tumor remnant was excised for examination. Subsequently there has been a tendency for gonadotrophin to rise; the chest plate and physical examination do not yet reveal a locus. Nitrogen mustard still has some inhibiting effect

on the growth, but does not eliminate it. A change to methotrexate may be made in the future.

This case is not presented as a therapeutic result as it is an unfinished study. The patient has lived much longer than one would have anticipated formerly. She looks and feels well and weighs 130 pounds, a striking contrast to the exsanguinated, 90-pound girl of more than 2 years ago. However, one must not be misled; she is living in uneasy symbiosis with her tumor. The case is presented because it illustrates the manner in which gonadotrophin assay increases understanding of phases of growth and response of these tumors to various events and agents.

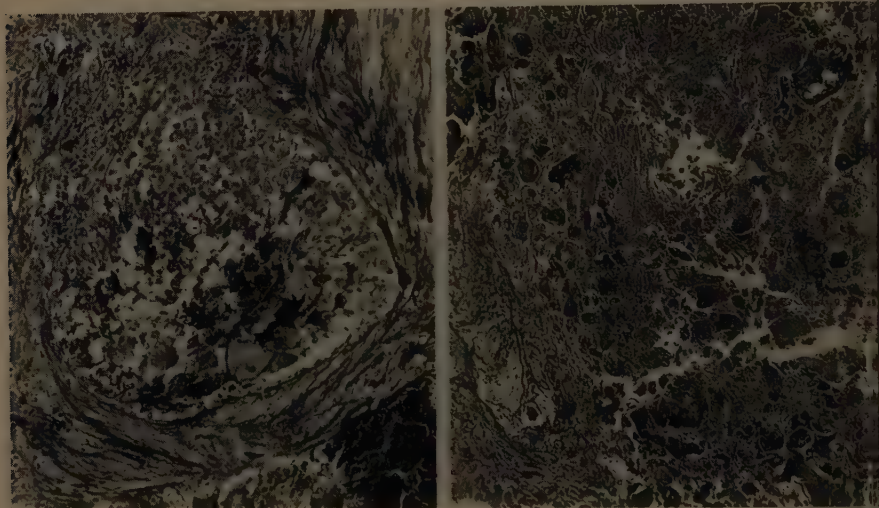


FIGURE 17. Case J. I. 13 24 60. Tumor 4 weeks after nitrogen mustard administration. Massive destruction is evident, but not uniform in all areas, as shown in two sections.

It should be noted that most of these cases that I have reported antedate the antimetabolites and chemical agents currently being used to inhibit tumor growth. For this reason they represent a background of behavior of such tumors against which one may project the response to these agents in the future.

Summary

The serum chorionic gonadotrophin has been studied in 129 patients with hydatidiform moles throughout their entire courses. Gonadotrophin production has been correlated with events in the natural history of the tumors and found to be a dynamic indicator of growth activity of trophoblast anywhere in the host. It provides a unique opportunity to follow the evolution of these tumors through phases of development that, in other tumors, are generally obscure. It is, moreover, an invaluable tool for clarification of clinical problems and evaluation of therapeutic results.

References

1. DELFS, E. 1941. An assay method for human chorionic gonadotrophin. *Endocrinology*. **28**: 196.
2. DELFS, E. 1957. Quantitative chorionic gonadotrophin. Prognostic value in hydatidiform mole and chorionepithelioma. *Obstet. & Gynecol.* **9**: 1.
3. JONES, G. E. S., E. DELFS & H. M. STRAN. 1944. Chorionic gonadotrophin and pregnanediol values in normal pregnancy. *Bull. Johns Hopkins Hosp.* **75**: 359.

Discussion of the Paper

H. G. GRADY: E. Delfs's paper emphasizes the great value of quantitative chorionic gonadotrophin determinations in diagnosis and clinical management of both mole and choriocarcinoma. The incidence of postmolar choriocarcinoma is somewhat higher in her cases than seems generally true in the United States, and may possibly be due to referral of an unusual number of severe cases.

R. W. NOYES: E. Delfs has shown many excellent case summaries with fluctuation in serum gonadotrophin levels following abortions or deliveries. I wonder how these patients came to be studied, as there is no indication that they were clinically ill at the time of the first analysis for gonadotrophin. I wonder if Delfs feels that there is a place for her gonadotrophin assay method in screening *post partum* and postabortal patients for the possible presence of chorionepithelioma.

HENRY A. THIEDE (*Rochester, N. Y.*): Have you observed any cases of proved choriocarcinoma when the chorionic gonadotrophin titer was negative? C. J. Lund of the University of Rochester, Rochester, N. Y., has a recent case in which toad tests were repeatedly negative in a patient with untreated choriocarcinoma proved by a subsequent hysterectomy.

E. DELFS: I have not seen a case of choriocarcinoma that was truly negative for chorionic gonadotrophin. The only exception was one patient who had a single negative assay shortly after a curettage; on a repeat assay three days later the titer was low but positive. The uterus showed only a tiny area of choriocarcinoma in its earliest stage. Probably gonadotrophin would have increased rapidly had the uterus not been removed at this point. I believe many of the so-called gonadotrophin-negative phases of choriocarcinoma depend upon quantitative factors: a small area of tumor, perhaps in an indolent growth phase, producing a relatively small amount of gonadotrophin. Some test animals such as the rabbit and toad are not very sensitive and may miss low titers, especially in urine which may be lower and less dependable than serum levels.

One may theorize that trophoblastic cells could become so disorganized as to lose their ability to perform the apparently basic function of chorionic gonadotrophin production. If this occurs, it must be quite rare, as I have not encountered it in more than fifteen years of assaying trophoblastic tumors with a sensitive test on blood serum.

Part III. Trophoblastic Tumors

THE ALBERT F. MATHIEU CHORIONEPITHELIOMA REGISTRY

John I. Brewer

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Albert F. Mathieu was born in 1881 and died in 1939. He became a member of the American Association of Obstetricians, Gynecologists and Abdominal Surgeons, Chicago, Ill., in 1929, and served as vice-president of that organization in 1937 and 1938. He was associate clinical professor of obstetrics and gynecology at the University of Oregon Medical School, Portland, Oreg. During his professional life, he contributed much to the literature of obstetrics and gynecology. His principal interests and writings were on chorionepithelioma, the most extensive of which was a collective review published in *Surgery, Gynecology and Obstetrics* in 1939.

In 1946, Albert W. Holman, who had worked with Mathieu in the studies on chorionepithelioma and hydatid mole, proposed to the American Association of Obstetricians, Gynecologists and Abdominal Surgeons that a memorial registry, to be known as the Albert F. Mathieu Chorionepithelioma Registry, be established by the association, and that he, Holman, would donate the sum of \$1,000 for its establishment. His motion was accepted and the registry was established at the final business session of the association on September 7, 1946. A committee was named, with Emil Novak as chairman, to begin the function of the registry, and an appropriation of \$200 was granted to the committee for the first year's work. Announcement of the establishment of the registry with its location at Johns Hopkins Medical School, Baltimore, Md., was published in various medical journals.

When a specimen is received, the chairman transmits his own diagnosis to the sender of the specimen as soon as possible. This is done more or less as a courtesy and to be helpful, since the registry was not founded as a diagnostic service laboratory. The case history and the microscopic slides are sent to the members of the committee in turn. The first to receive the material records his diagnosis, sends the material to the next member, and transmits his diagnosis to the chairman. Each member performs the same procedure, and the last to receive the material returns it to the chairman. The chairman then reviews the material if there are variations in diagnoses, attempts to resolve the differences, and reports to the original sender of the specimen the final conclusions of the committee. The chairman sends to each member of the committee a list of the diagnoses made by all the members. A copy of this list is attached to each case history. The chairman selects a single diagnosis (often rather empirically) for the purposes of classification and filing. A follow-up on all living patients is made every year.

In 1948, at the annual meeting of the association, Emil Novak, chairman of the Chorionepithelioma Committee, reported that a large proportion of the cases received as chorionepithelioma, or suspected of being chorionepi-

thelioma, had proved to be examples of histologically benign or, perhaps, proliferative types of moles. Some were instances of nonneoplastic syncytial endometritis. From the standpoint of accuracy in diagnosis, the value of a central registry was stressed and was apparent to all. The committee utilized these classifications: (1) benign hydatid mole, (2) malignant or potentially malignant mole, (3) chorionepithelioma, and (4) syncytial endometritis.

A discussion by the Fellows of the association was held concerning the possibility of joining the Albert F. Mathieu Chorionepithelioma Registry with a national registry of obstetrical and gynecological pathology to be conducted by the Army Institute of Pathology, Washington, D. C. Such a union was rejected by a vote of the Fellows.

By 1949, there were 85 cases in the registry, of which 21 were authentic chorionepitheliomas. Specimens that were not chorionepithelioma were extremely valuable to the registry; therefore Novak made a plea for receipt of all types of pregnancy material, both benign and malignant. Forty-one cases of benign hydatid mole were registered. At this annual meeting in 1949, Novak presented a paper entitled "Pathology Aspects of Hydatidiform Mole and Choriocarcinoma," for which much of the material was culled from the registry.

In 1950, Novak stressed again that the registry did not purport to offer a rapid diagnostic service, but that its chief function was the accumulation and authentication of submitted material with the objective of later large-scale studies that might be expected to throw light on the clinical and pathological characteristics of chorionepithelioma and hydatidiform mole. A total of 140 cases was registered at the end of 1950. At this meeting in 1950, Novak requested, and was granted, a budget of \$250 yearly.

By 1952 the registry contained 200 cases: 62 chorionepitheliomas, 87 hydatid moles, 10 choriadenoma destruens, and 26 syncytial endometritis. Novak reported that the original grant of \$1,000 from Albert Holman had maintained the registry until 1952. In 1952, the American Cancer Society, Inc., New York, N. Y., awarded a grant of \$500 to the registry. The committee urged the association to search and obtain for the registry a more permanent form of financial support.

At the annual meeting in 1953, it was announced that the Obstetrical and Gynecological Assembly of Southern California, Los Angeles, Calif., would assume this responsibility through an annual grant to the association of \$1,500. At this annual meeting Novak and C. S. Seah presented the choriocarcinoma material from the registry in a formal paper entitled "Choriocarcinoma of the Uterus: A Study of 74 Cases from the Mathieu Memorial Chorionepithelioma Registry." At the business session of the association a discussion concerning the merging of all tumor registries was held. By action on a formal motion, the fellowship voted that the status of the Albert F. Mathieu Chorionepithelioma Registry should remain unchanged and that it should not be merged with other registries.

During 1954, the total cases in the registry reached 330, of which 87 were choriocarcinoma and 145 benign hydatid moles. The expenses of the registry had increased to \$830. The grant of \$1,500 from the Obstetrical and Gynecological

cological Assembly of Southern California again made it possible for the registry to function. Three publications from the registry appeared in 1954 in the *American Journal of Obstetrics and Gynecology*.

In 1955, welcome word was received from the Obstetrical and Gynecological Assembly of Southern California that the sum of \$1,500 a year would be granted for the next 3 years. Novak reported the diagnoses of 390 completed cases and noted that there were 25 additional cases in the process of completion, making the grand total of 415. The expenses were \$872.

In 1956 the completed cases numbered 440. There were 20 uncompleted cases, making the total registered 460. There were 125 choriocarcinomas and 200 moles, and the remainder were other types of trophoblastic abnormalities. The expenses were \$1,000.

In 1957 Novak died. The work in his laboratory on the registry material was carried on most efficiently by his son, Edmund R. Novak and J. Donald Woodruff until the end of the year. At the end of 1957 there were 500 completed cases. The expenses were \$890. At the annual meeting in 1957, the American Association of Obstetricians and Gynecologists (the name was changed from the American Association of Obstetricians, Gynecologists and Abdominal Surgeons in 1954) awarded the registry to the Department of Obstetrics and Gynecology of Northwestern University Medical School and stated that the transfer of material and records should be effected January 1, 1958. I was designated chairman of the committee to carry on the work of the registry.

During 1958, 37 new cases were added, bringing the grand total to 537, of which 147 are choriocarcinoma and 240 are benign moles. A grant of \$5,500 was requested and received from the American Cancer Society, Inc., New York, N. Y., for a special project that is now in progress. In addition to this project, there are 4 other studies in progress.

In the 12 years the registry has been in existence, specimens have been received from nineteen countries other than the United States, which indicates the interest the registry has developed. Much of this was due to the reputation of the first chairman, Emil Novak. We are indebted to him, and to Albert Holman for their efforts in the development of a most valuable registry.

CHORIOADENOMA DESTRUENS*

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Introduction

This paper contains only a few references to the literature and consists primarily of the results of a study of chorioadenoma destruens in the material of the Mathieu Memorial Registry of Chorionepitheliomas of the American Association of Obstetricians and Gynecologists, Chicago, Ill.

Definitions

The term chorioadenoma destruens was first proposed by Ewing (1910) who, as I understand it, intended this term to include moles that invade into the uterine wall and/or metastasize and still maintain their villous structure. Hertig and Sheldon (1947) consider a chorioadenoma destruens an "invasion of myometrium by malignant mole" and believe that these almost never metastasize. Novak and Seah (1954) define the term somewhat differently. They include as chorioadenoma destruens those moles in which there is (1) "an inordinate degree of trophoblastic proliferation" and (2) "undue penetration of the mole through the uterine wall, into the peritoneal cavity, the parametrium, or the vaginal vault." According to their first definition there is no need for invasion or metastasis, since the diagnosis is made solely on the basis of marked proliferation of trophoblastic elements. Thus the diagnosis can be made from a dilatation and curettage specimen only. Actually, in the material cross-filed in the Registry as chorioadenoma destruens, 2 such specimens are included.

I believe Ewing's definition to be preferable. Specifically, there must be either invasion by a mole into or through the uterine wall and/or metastasis, and the metastasis must contain hydatidiform villi. The diagnosis of chorionepithelioma is justified when large erosive masses of trophoblastic cells are found in the uterine wall or as metastases and contain no villi. As previously stated, two diagnoses of chorioadenoma destruens were made from curettement specimens only. One of these I have omitted. While it did show a marked proliferation of trophoblastic elements, there was no evidence of invasion of myometrium. The second I have included. This specimen resulted from a very heavy-handed curettage, to say the least. There were large masses of myometrium included with the specimen and in several areas there was obvious invasion deeply into the muscle of hydatidiform villi with proliferation of trophoblastic elements. One shudders to think how close the operator in this instance came to tearing through the wall of the uterus.

* The work reported in this paper was supported in part by a cancer control grant from the National Cancer Institute, Public Health Service, Bethesda, Md.

Material

Sixty-two examples of chorioadenoma destruens are listed in the Registry. Limited to the period from 1947 through 1956, however, 55 are listed. Only the latter are included in this study; the others have been omitted since too little time has elapsed to allow for follow-up. Of the remaining 55, 13 have not been included in this study for a variety of reasons. In two there was no follow-up. One (a dilatation and curettage specimen) is considered to represent only an unusually active-appearing hydatidiform mole. Four were omitted because no villi were present, and I consider that the proper diagnosis in these cases should have been chorionepithelioma. In 5 instances the slides were either totally inadequate for study or slides were missing. In 1 instance the case record apparently had been lost. There thus remained 42 chorioadenoma destruens.

The diagnosis was made from hysterectomy specimens with or without metastases in 35 instances. The diagnosis was made from vaginal or vulvar metastasis without having a hysterectomy specimen in 4 instances. As previously mentioned, the diagnosis was made once from a curettage specimen only. Another was made from a salpingectomy specimen in which the mole had perforated through the wall of the tube. The final specimen consisted of the excised tract of a perforation through the uterine wall.

Deaths

Six deaths occurred. The first (C.R. No. 97) was referred to in the publication by Novak and Seah. This patient had a local recurrence in the pelvis and, five months after hysterectomy, a radical cleaning out of the anterior portion of the pelvis was done with implantation of the ureters into the sigmoid colon. Six weeks later this patient died of uremia.

The next patient (C.R. No. 139) developed a local recurrence and also metastases in the lung. She died 15 months posthysterectomy. In the hysterectomy specimen a markedly invasive lesion was present in the myometrium with relatively tremendous proliferation of the trophoblasts. In some areas villi were not present. This patient was reported as a choriocarcinoma by Novak and Seah. Since villi were present in the lesions in the uterine wall, the proper designation seems to me to be chorioadenoma destruens. At autopsy tumor tissue was reported in the lungs, brain, and pelvis. Unfortunately these slides were not available for study, so it is not known whether metastases were still chorioadenoma destruens or were choriocarcinoma.

The next (C.R. No. 202) was referred to in the publication by Novak and Seah as a chorioadenoma destruens. She developed a cord lesion and died as a cachectic paraplegic nine months posthysterectomy. Unfortunately no autopsy was done, so it is not certain whether this represents a metastasis from her chorioadenoma destruens.

The next (C.R. No. 290) is equally unsatisfactory. She had a hysterectomy in December 1952. The hysterectomy specimen showed invasive mole.

She died on July 12, 1953. The cause of death was not given and no report was made of any X rays of the lungs.

The next (C.R. No. 316) died 33 months posthysterectomy from lung metastasis. The hysterectomy specimen showed an unusually active invasive mole. At autopsy the tumor was reported to be present in lungs, liver, and spleen. Unfortunately these slides were not available for examination. It is thus not known whether this tumor metastasized as a chorioadenoma destruens or as a choriocarcinoma.

The next (C.R. No. 410) died from lung metastases 11 months posthysterectomy. These metastases contained villi and obviously represented metastatic chorioadenoma destruens and not choriocarcinoma.

Vulva and/or Vaginal Metastases

In 6 of the 42 patients there were metastases to the vulva and/or vaginal mucosa. In 1 of these (C.R. No. 70) there was also local recurrence postoperatively in the pelvis and lung metastases. This patient was treated by dilatation and curettage, excision of a local lesion, and deep X-ray therapy to the lungs and pelvis. The metastatic lesions underwent regression and she was living and well 9 years later. The next patient (C.R. No. 80) had a lesion in the vaginal wall that was excised at the time of hysterectomy. She was living and well 5 years later. The third such patient (C.R. No. 170) had a large lesion of the anterior vaginal wall extending up under the bladder. It was impossible to excise this lesion completely. Following total hysterectomy and bilateral salpingo-oophorectomy, deep X-ray therapy was given to the pelvis. The lesion regressed and she was living and well 6½ years later. The next patient (C.R. No. 286) had a large fungating mass filling the vagina and extending from urethra to cervix. This was biopsied and she was given testosterone and deep X-ray therapy. She was living and well 5 years later. The next (C.R. No. 372) had a vaginal nodule that was excised. This contained a typical hydatidiform mole tissue. Unfortunately the uterus, which was removed, was not subjected to histopathological study. The patient, however, was living and well 2 years following surgery. The final patient (C.R. No. 450) had a hemorrhagic mass in the vaginal wall. This was excised and she was subjected to hysterectomy. Six months later she was living and well, with a negative chorionic gonadotrophin test.

Lung Metastases

Lung metastases were definitely present in 6 patients and, questionably, in a seventh. The first patient (C.R. No. 65) developed lung metastases which, however, underwent spontaneous regression. Eight years later she was living and well and had had 2 babies in the meantime. The next (C.R. No. 70) has already been referred to under *Vaginal Metastases*. The next (C.R. No. 139) has been discussed under *Deaths*. The next (C.R. No. 213) had questionable lung metastases. The roentgenologist interpreted the findings as demonstrating a lung metastasis, but the gynecologist disagreed. Nevertheless, 5 years later, the patient was living and well. The

next (C.R. No. 263) showed multiple metastatic lesions in the lung in the postoperative X ray. There was a slight increase in size of the lesions in the immediate postoperative period but, 18 months later, the lungs were completely clear. The patient was living and well 3 years later. The remaining 2 with lung metastases (C.R. No. 316 and No. 410) have already been discussed under *Deaths*.

Local Recurrence

Local recurrence in the pelvis developed in 6 patients and, questionably, in another patient. The first (C.R. No. 70) has already been referred to under the heading *Vulvar and Vaginal Metastases*. The next (C.R. No. 97) has been referred to under the heading *Deaths*. The next (C.R. No. 149) had a local recurrence in the pelvis which underwent spontaneous regression. She was living and well 7 years later. The next patient (C.R. No. 139) with local recurrence in the pelvis also had lung metastases. She has been discussed under the section *Deaths*. The next (C.R. No. 249) had a local recurrence in the pelvis. She was given deep X-ray therapy and the lesion regressed. She was living and well 5 years later. The last (C.R. No. 405) developed a local recurrence in the pelvis which was reoperated 20 months posthysterectomy. Unfortunately the results of this latter operation and further follow-up on this patient are not available.

Relation of Trophoblastic Activity to Perforation, Local Recurrence, and Metastases

The relative degree of trophoblastic proliferation was tabulated for 38 of the cases. It was believed that this would give a fairly accurate index of the activity of the lesion and, perhaps, might indicate the future prognosis as to whether the lesion would perforate through the uterus, recur locally, or metastasize. The tabulation was made first with those that had perforated through the uterus, recurred locally, or had metastasized. These findings were quite impressive. It seemed that there was a high percentage of active-appearing moles in this group. It developed that 9 showed a marked to extreme degree of trophoblastic activity, 9 showed a moderate degree of trophoblastic activity, and 5 showed a slight to inactive degree of trophoblastic activity. Unfortunately, within the limits of this small series the same thing proved true when the findings were tabulated for those that had not perforated through the uterus, did not locally recur, and had not metastasized. Nine showed marked or extreme activity. Four showed moderate activity and 2 slight to relatively no activity. When the tabulation finally was limited to those cases that either metastasized or locally recurred, 6 showed marked to extreme activity, 7 showed moderate activity, and 3 slight to relatively no activity: certainly no different from those that were only locally invasive in the uterine wall. In the 6 patients that died the relative degree of activity of the mole was also approximately the same as in the nonperforating, nonmetastasizing group: 3 showed marked activity, 2 moderate, and 1 slight.

Comments

Chorioadenoma destruens is often referred to as the benign tumor of the trophoblasts. From some of the data that have been presented I do not feel that the word benign is quite applicable. Certainly in Case No. 410, in which the mole metastasized as a mole to the lung and killed the patient, it was not a benign tumor. Unfortunately, in the other two cases that definitely died of lung metastases it is not known whether the metastases were choriocarcinomas or chorioadenoma destruens. However, when the diagnosis is made of chorioadenoma destruens from a hysterectomy specimen or from a metastasis to the vagina or vulva, happy complacency does not seem to be exactly in order. The patient should be followed with great care with repeated X-ray examinations of the lungs and repeated chorionic gonadotrophin tests (not that these procedures will alter the course of the disease).

It appears that lung metastases following chorioadenoma destruens in the uterus *tend* to regress spontaneously or else to respond to X ray. This has been noted by others, including Hunt *et al.* (1953), Friedman (1957), Nolan *et al.* (1950), and Bardawil *et al.* (1957). Unfortunately, this tendency is just a tendency and does not always occur. In this series definite metastases were found in the lung in 6 instances (omitting C.R. No. 213). Three patients died, 3 survived. Regression of the lung lesion was spontaneous in 2 and followed X-ray therapy in 1.

Metastases to the vagina and/or vulva in this small series have not had such an ominous implication. Such metastases were present in 6 patients. In 1 (C.R. No. 70), metastases were also present in the lungs. Her lesions regressed following excision and X ray. Three others were treated by simple excision (as well as hysterectomy) and were cured. Another was treated by excision, hysterectomy, and deep X ray and was cured. In the sixth patient the lesion could not be completely excised, but was cured by deep X-ray therapy.

Definite metastases to one site or another occurred in 11 of the 42 patients, or in 26 per cent. Possible metastases occurred in 2 more patients. If these were included, the percentage would increase to 30.5.

Definite local recurrences appeared in 6, or 14.3 per cent of the 42 patients with chorioadenoma destruens. Two of these also had lung lesions; 1 of these died. Of the remaining 4, 1 died following radical surgery and 1 was reoperated 20 months posthysterectomy with unknown results. In one patient there was spontaneous regression and, in another, the lesion disappeared following X-ray therapy.

The over-all mortality was 6 of 42 cases, or 14.3 per cent, that could be studied adequately. This figure represents a high mortality for a "benign" disease. Park and Lees (1950) tabulated 47 cases of invasive moles from the literature. Seventeen of the 47 proved fatal, 2 with definite metastases as choriocarcinoma, 1 probable choriocarcinoma, 1 possible choriocarcinoma, 1 of unknown cause, 1 with ruptured uterus, 1 from external hemorrhage, 3 from intraperitoneal hemorrhage, 1 from peritonitis, and 6 immediately

postoperatively. Park and Lees do believe, however, that there was no more chance of a choriocarcinoma developing from an invasive than from a noninvasive mole. Nevertheless, the 36 per cent over-all mortality is impressive, even though one must grant that with modern-day blood banks and antibiotics it would have been lower.

On the other hand, the presence of metastasis does not mean a uniformly fatal outcome. In 11 instances of definite metastases (excluding C.R. No. 202 and No. 213) to any area, death ensued in 3 cases, or in 27 per cent. If the metastases were to the lungs, death ensued in 3 of 6 cases, or in 50 per cent.

Summary

Forty-two cases of chorioadenoma destruens from the Mathieu Chorionepithelioma Registry of the American Association of Obstetricians and Gynecologists have been studied in detail. Cases have been limited to those in which there was invasion of the uterine wall and/or metastases that contained hydatidiform villi.

Metastases occurred definitely in 11 and questionably in 13. Definite metastases to the lung occurred in 6. Three of these patients lived and 3 died. In one of the latter instances the lung metastases contained molar tissue.

Definite local recurrences developed in 6 patients. In 1 the local recurrence can be considered as at least the indirect cause of death. The status of another who had to be reoperated for local recurrence is not known.

The over-all mortality was 6 of 42 patients, or 14.3 per cent. This is considered to be rather high for a lesion whose name implies benignancy.

References

1. BARDAWIL, W. A., A. T. HERTIG & J. T. VELARDO. 1957. Regression of trophoblastic I. Hydatidiform mole; a case of unusual features. *Obstet. & Gynecol.* **10**: 614-623.
2. EWING, J. 1910. Chorioma. *Surg. Gynecol. Obstet.* **10**: 366-392.
3. FRIEDMAN, J. R. 1957. Hydatidiform mole with metastases. *J. Kentucky State Med. Assoc.* **55**: 1001-1006.
4. HERTIG, A. T. & W. H. SHELDON. 1947. Hydatidiform mole—a pathologicoclinical correlation of 200 cases. *Am. J. Obstet. Gynecol.* **53**: 1-36.
5. HUNT, W. M., P. DOCKERTY & L. M. RANDALL. 1953. Hydatidiform mole. *Obstet. & Gynecol.* **1**: 593-609.
6. NOLAN, J. F., E. M. COOK & W. E. DALKE. 1955. Metastatic pulmonary chorionepithelioma with recovery in two cases. *West. J. Surg.* **63**: 101-106.
7. NOVAK, E. & C. S. SEAH. 1954. Choriocarcinoma of the uterus. *Am. J. Obstet. Gynecol.* **67**: 933-961.
8. PARK, W. W. & J. C. LEES. 1950. Choriocarcinoma. *A. M. A. Arch. Pathol.* **49**: 73-104; 205-241.

Discussion of the Paper

H. G. GRADY: R. Greene's careful evaluation of the cases of chorioadenoma destruens from The Albert Mathieu Chorionepithelioma Registry is of great importance, for we have greatly needed accurate information of the behavioral history of this intermediate lesion. Certainly, the old belief that a trophoblastic lesion possessing villous structure would probably not metastasize

size has been thoroughly corrected by the results of this study. This lesion is shown also to be a much less malignant if not benign process than is choriocarcinoma. If the term chorioadenoma destruens is to be decently interred, as all seem to agree it should be, then, on the basis of these papers, invasive mole seems to be its legitimate successor.

H. W. EDMONDS: I am impressed by the fact that R. R. Greene has shown that his grading of the trophoblast of the invasive moles in his series did not tally with the aggressiveness of the moles as indicated by either perforation or deportation. This should strengthen our reservations concerning any of the existing schemes for grading hydatidiform moles solely on the basis of morphology of their trophoblast.

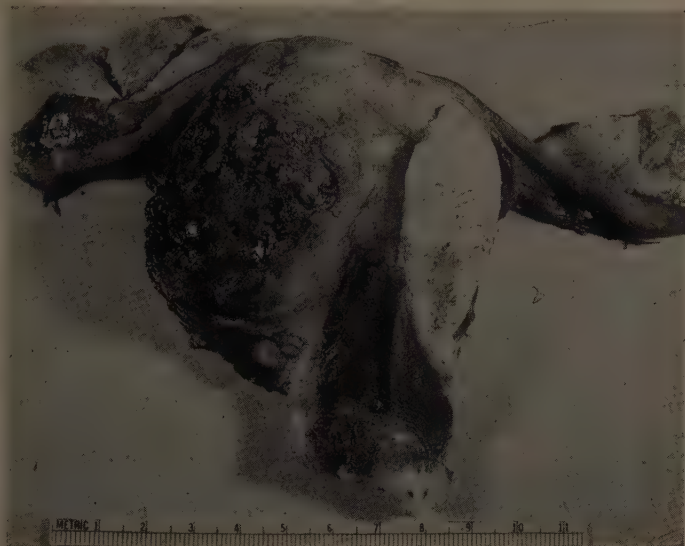


FIGURE 1

Since an invasive mole is really nothing more than *any* mole that for some reason has penetrated to a greater than average depth, I have speculated that simple penetration of a gynecologist's curette into or through the myometrium, in an accidental perforation, would suffice to change an ordinary mole into an invasive one by carrying molar tissue into or through the muscle. I wonder if anyone else has recognized such a situation.

NATHAN B. FRIEDMAN (*Cedars of Lebanon Hospital, Los Angeles, Calif.*): I have encountered one mole under circumstances that support one of H. W. Edmonds's suggestions. The patient had a curettage for mole and subsequently a hysterectomy. An invasive mole occupied the subserosal myometrium and a sinus tract led into it from an endometrial defect (FIGURE 1). Incidentally, the great efforts made to avoid a hysterectomy in dealing with a mole stand in striking contrast to the lack of reluctance in performing the same procedure for conditions which present no hazard to the patient.

One might receive the erroneous impression from the literature and the discussions elsewhere in these pages that the behavior of trophoblastic tumors is basically different from that of neoplasms in general. One of the normal functions of trophoblast is to invade; another, to regress. Therefore, it should not be surprising that a growth of this tissue can be invasive and still be benign, or that it can regress and still be malignant. The evaluation of infiltration in many neoplasms is difficult enough without the added problems created by the normal invasiveness of trophoblastic tissue. Many other malignant tumors occasionally regress. Papillary carcinomas of the thyroid, ductal carcinomas of the breast in association with Paget's disease of the nipple, and Kaposi's sarcoma come to mind, to say nothing of ovarian and hypernephroid neoplasms.

M. W. ORSINI (*Department of Anatomy, University of Wisconsin, Madison, Wis.*): The cells shown in R. R. Greene's "ringer" are extremely similar to those found in the *post partum* hamster. These I believe, from developmental studies, to be of trophoblastic origin and not of endothelial origin. Use of the sex chromatin technique is of no avail to settle this question in the hamster, since the sex chromatin is not visible in laboratory rodents. However, J. Dixon Boyd, of the University of Cambridge, Cambridge, England, and W. J. Hamilton, Charing Cross, England, told me in the summer of 1956 that by use of sex chromatin in the human, they had been able to show that some, at least, of these cells within the maternal arteries are fetal. At that time they had only three cases. This is to be quoted in Transactions of the Fifth Conference on Gestation, 1958, at Princeton, N. J. sponsored by the Josiah Macy, Jr. Foundation.

WILLIAM B. OBER (*Knickerbocker Hospital, New York, N. Y.*): There is considerable variance in opinion regarding the nature of chorioadenoma destruens. Some observers feel that it is not even a true tumor and call it an invasive mole or mola destruens. Others feel that it achieves the dignity of a true tumor and label it a chorioadenoma destruens, that is, a locally destructive benign tumor of chorionic epithelium. Still others, such as R. R. Greene, feel that it is not so benign as all that. I am inclined to argue that the mere fact that a patient with chorioadenoma dies of the disease does not mean that it is truly a malignant tumor. Fatality can occur with benign tumors in other situations. I think it is important to be rigid, even catatonic, when classifying proliferations of a tissue that, even when clearly benign, has the capacity to invade and to embolize. However, precision in histologic evaluation should not blind one to the fact that chorioadenoma destruens has a certain range of possible behavior. Like any other tumor, some cases are more aggressive than others, while some may be relatively indolent.

The decisive proof of malignancy in chorionic growths has to be invasion of the parenchyma of maternal organs. I am not impressed with so-called vaginal metastasis as a sign of malignancy; I feel that this is largely a permeation of dependent venous channels by intravascular trophoblast and that true infiltration of maternal tissue rarely occurs. The granulation tissue in such lesions merely represents the response of the maternal organism to

trophoblast. While this reaction adds bulk and a sense of mass to the lesion, it does not imply that the trophoblast has gone beyond vascular confines. Likewise, X-ray evidence of pulmonary metastasis in cases of chorioadenoma destruens may be misleading. The shadows may be due to a reaction in lung tissue around embolic trophoblast with or without villi, but not of necessity due to trophoblast invading maternal lung tissue.

Applying these rigid criteria at two removes, I am inclined to accept only 3 of Greene's 6 fatal cases as showing metastases from a chorioadenoma destruens (C. R. Nos. 139, 316, and 410). In the series that Park and Lees (quoted by Greene) culled from the literature, there were 47 cases of invasive mole with 17 deaths and with proven metastasis in 2 cases, probable in 1, and possible in 1. As a sporting venture, I accept the 1 probable case and discount the possible case. In the classic series of 200 moles reported by Hertig and Sheldon, there were 32 examples of chorioadenoma destruens with 1 death due to sepsis and no metastasis. In an unpublished series, A. B. Little informs me that he and his colleagues have at least 21 cases of chorioadenoma destruens with only 1 fatality due to metastasis. A bit of quick arithmetic enables us to total these 4 series. Out of a total of 142 chorioadenomata destruents, there are 25 deaths (17.6 per cent) and 7 cases with proved metastases (4.9 per cent). This figure is considerably lower than that based on Mathieu Registry cases alone. Possibly it reflects the fact that unusual cases tend to be sent to registries, whose files are a concentrate of the population at large. It is only fair to state that a death rate of 17.6 per cent is high for this day and age. More than two thirds of the 25 fatal cases are those that Park and Lees summarized from the literature, many of them dating back to a period when hemorrhage and sepsis were treated less effectively than today. If we take the two more recent series of Little *et al.* and of Hertig and Sheldon, there are 53 cases with 2 deaths (3.8 per cent) and 1 case with metastasis. On this basis chorioadenoma destruens may be regarded as dangerous; it requires hysterectomy. Serious sepsis and serious hemorrhage may occur. However, it very rarely metastasizes and, for the most part, it behaves like a benign tumor.

ASPECTS OF CHORIOCARCINOMA IN THE FEMALE

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The Histological Diagnosis of Choriocarcinoma

To diagnose a choriocarcinoma before the patient is *in extremis* or dead is never easy. Sometimes the behavior of pregnancy test titers or radiological appearances in the lungs allow a virtually certain diagnosis before the patient is gravely ill, but such cases are few; diagnosis otherwise and, indeed in almost every case, is wholly dependent upon the microscope.

The histological diagnosis of any cancer depends upon the fact that certain abnormalities of tissue pattern, from past experience, are associated in a high proportion of cases with progressive growth of the abnormal tissue and the death of the patient, certainly in the absence of treatment and, very often, even after treatment. The closer is this association, the more reliable the diagnosis. It is obvious that the first step in establishing a diagnosis of cancer is the accurate identification of the abnormal tissue pattern as a pattern whose association with subsequent cancerous behavior is already established on past knowledge; with most tissues this is relatively straightforward. Diagnosis then has a high degree of accuracy, and figures for incidence of the cancer and its curability are correspondingly dependable. This reliability is attested by the very close similarity of reported figures for the incidence and curability of common cancers, such as those of breast and stomach, in various clinics. With choriocarcinoma, on the other hand, the situation is different. Wide differences in the reported figures of incidence and curability in different clinics raise doubts whether in trophoblast a histological pattern actually diagnostic of cancer exists. There is no doubt in the highly characteristic appearance of malignant trophoblast obtained from the patient dead of choriocarcinoma: once seen, never forgotten, even by students in a class of pathology. The problem, however, is: can tissue of the same appearance be present within the uterus without necessarily presaging metastasis and death?

One of the best ways of determining whether there exists a histological pattern specific for any particular type of cancer is to submit the same sections of possible examples of the cancer to several pathologists and to analyze the results in order to learn: first, whether consistency of interpretation can be achieved; and, second (if a satisfactorily high degree of consistency has been achieved) whether a significantly high proportion of the patients later die of the cancer, thus confirming the correctness of the interpretation. The greater the number of cases that can be examined thus the more critical and helpful will be the proof. To be complete, an analysis of this kind obviously requires knowledge of the fate of the patients. However, even if this knowledge be scant and the absolute correctness of

attempted diagnosis thus difficult to assess, one can still assess at least the consistency of attempted diagnosis. A fully comprehensive analysis of this kind is not always easy to arrange, especially with so rare a lesion as choriocarcinoma, but fortunately The Albert Mathieu Chorionepithelioma Registry, Chicago, Ill., provides material eminently suitable for assessing the consistency of histological interpretation and, in part also, for assessing its absolute correctness. In almost every case in that collection (which, of course, contains cases of simple hydatidiform mole as well as cases of choriocarcinoma and possible choriocarcinoma) the microscopic sections have been submitted to members of a committee of examiners for their individual opinions, and these opinions have been recorded in the case files. It is on some of this material that an analysis has been made with a view to determining the reliability of the histological diagnosis of choriocarcinoma.

For the purpose of this analysis the recorded opinions on 200 consecutive cases were used (Nos. 186 to 390, omitting 5 that were unsuitable: No. 189, ovarian pregnancy; No. 269, tissue inadequate; No. 277, 1 opinion only; No. 285, carcinoma of ovary; No. 364, choriocarcinoma testis). There were 5 independent opinions in 54 cases, 4 in 144 cases, and 3 in 2 cases. Their distribution is shown in TABLE 1. By agreement is meant that all the exam-

TABLE 1

ANALYSIS OF HISTOLOGICAL OPINIONS ON 200 CASES OF TROPHOBLASTIC ABNORMALITY
(5 OPINIONS IN 54 CASES; 4 IN 144 CASES; 3 IN 2 CASES)

Agreement on: 74 cases of simple hydatidiform mole

10 cases of simple abortion

27 cases of choriocarcinoma (17 accompanied by sections of metastases)

Partial disagreement on:

27 cases—hydatidiform mole or not (?)

19 cases in varying degree "suspicious," mostly hydatidiform moles

Disagreement (one or more opinions "for" choriocarcinoma, others "against") on:

43 cases

That is, 89 cases where question of choriocarcinoma arose

ning microscopists concurred in the diagnosis. In 27 of the 46 cases of partial disagreement the disagreement reflected no more than differences in criteria for the diagnosis of a simple hydatidiform mole; in view of the relative frequency with which hydatidiform villi may be seen in aborted material (what may be called a hydropic abortion) this is not unexpected. The other 19 cases in this category chiefly concerned hydatidiform moles that were regarded by some of the committee as simple and by others as suspicious. In the 43 cases where there was disagreement, one or more of the committee had said choriocarcinoma or malignant when others had said in effect not malignant (for example, benign molar residues, chorioadenoma destruens, syncytial endometritis, abortion).

It will be clear that in the 74 cases of simple hydatidiform mole, 10 of simple abortion, and 27 of hydatidiform mole or not (?) the question of choriocarcinoma was not at issue. In the remaining 89 cases the question of choriocarcinoma did arise, and the distribution of these cases is shown in TABLE 2. In 19 cases (21 per cent) there was neither unanimity of opinion

nor complete disagreement upon the significance of the histological appearances. Usually the difference of opinion was whether the lesion was a simple hydatidiform mole or whether it should be called a malignant mole or chorioadenoma destruens. In other cases the verdicts included such phrases as possibly malignant and probably choriocarcinoma. With a tissue as difficult to assess as aberrant trophoblast it is not surprising that a borderline group such as this should emerge during the analysis; indeed, it is an indication of the honesty of the histological verdicts.

TABLE 2

89 CASES WHERE QUESTION OF CHORIOCARCINOMA AROSE

Agreement on: 27 cases of choriocarcinoma (17 accompanied by sections of metastases) = 30 per cent
Partial disagreement on: 19 cases in varying degree "suspicious" = 21 per cent
Disagreement on: 43 cases = 48 per cent
Of the 43 cases: in 6, curettings only available
in 35, sections from the excised uterus
in 1, a vaginal node + curettings
in 1, a vaginal node only

In 27 cases (30 per cent) all the examiners had agreed that the tissue pattern was that of choriocarcinoma. There is a slight chance that this figure of 30 per cent may be artificially high for, in 17 of the 27 cases, the sections of the primary lesion were accompanied by sections of its metastases; the simultaneous presence of sections of metastases always introduces at least some risk of bias in favor of a diagnosis of malignancy. The figure of 30 per cent at any rate is not likely to be artificially low, and probably represents a maximum value for the chances of being able to diagnose choriocarcinoma with virtual certainty in any given case. In the remainder of these 89 cases where the question of choriocarcinoma arose, in almost one half (48 per cent) the same histological patterns had been interpreted in opposite ways by one or more of the examiners. It was possible to analyze this particular group a little more finely by noting the fate of the patients in those cases where only one opinion was at variance with the others for, as remarked previously, the fate of the patient is obviously the ultimate criterion by which the validity of diagnosis can be judged.

There were 21 such cases. In 14 there were either 3 or 4 diagnoses of non-malignant to one of malignant: 8 of these patients were alive for 17 months or longer; 2 were dead; and 4 not traced (when the analysis was done). In 7 there were either 3 or 4 diagnoses of malignant to 1 of not malignant: 4 of these patients were dead; 1 was alive 8 years later; and 2 were not traced.

There was thus a distinct tendency for the majority verdict to be correct, suggesting that the chances of establishing a correct histological diagnosis may be considerably higher than the 30 per cent noted earlier. On the other hand, this possibility might well be offset by the already-mentioned risk of bias in the opposite direction introduced into the group of unanimously malignant cases by the sections of metastases that accompanied 17 of them. TABLE 2 also shows that, in 35 of the 43 cases where there was disagreement

on the question of malignancy, sections from the excised uterus were available for examination. This shows clearly that difficulty in the assessment of abnormal trophoblast is not confined to the examination of uterine curettings alone.

The main conclusion that may be drawn from this analysis is that trophoblast has a much wider range of biological than of histological behavior; or, to express the matter in another way, the wide range of biological behavior of trophoblast is not reflected in a correspondingly wide range of histological structure, or, at least, not of a kind that can be detected with present techniques. There is little doubt, I think, that trophoblast does possess as wide a range of biological behavior as do most other epithelial tissues (it is difficult otherwise to explain the remarkably sessile or nonmetastasizing character of the highly invasive mola destruens or chorioadenoma destruens) and, in the absence of parallel histological changes, we are unable to establish the all-important correlation between histology and clinical course on which the whole of the microscopic diagnosis of malignancy depends. The microscope may be the ultimate arbiter in establishing the diagnosis of choriocarcinoma, but only for want of a better means, and we do well to remember that as an arbiter it is not infallible.

Trophoblast as a Graft

In the vocabulary of the plastic surgeon, gestational trophoblast, the tissue of another individual, is a homograft. However, grafting in the human is ordinarily successful only with the patient's own tissues; such grafting is known as an autograft. Homografts can be successfully exchanged between monozygotic twins, but otherwise the life of a homograft is no more than 10 to 15 days (corneal grafts are a noteworthy exception). After this time they suffer progressive deterioration and are finally cast off; to this gradual and ultimately complete disintegration the term homograft reaction is applied. This brief union of a mere fortnight compares interestingly with the reception accorded to gestational trophoblast. Since this tissue is normally retained in the human for 40 weeks it is clearly a homograft that enjoys a quite unusually long survival. In view of the increasing interest now being taken in tissue transplantation in general, and its immunological implications in particular, it is surprising that so little attention has been paid to trophoblast in this connection. However, a recent article by Doniach *et al.* (1958) discusses graft-immunity aspects of choriocarcinoma with particular reference to the use of immunizing therapy in a patient with choriocarcinoma. The general approach to the matter has since received editorial comment in *The Lancet* (1958).

The reason for the unusually long survival of trophoblast qua homograft has yet to be explained satisfactorily. A long series of observations by Medawar and his colleagues has shown that, in lower animals at any rate, the homograft reaction can be accelerated or prolonged or even abolished altogether. Thus Billingham *et al.* (1956) have shown that, if an animal is grafted on two successive occasions with skin from the same donor, the survival of the second graft will be significantly shorter than that of the first

graft (this intensification or acceleration of the homograft reaction is also demonstrable in the human). Abolition of the homograft reaction, on the other hand, can be achieved by exposing an animal in fetal life to cells from its future donor or from another member of the donor's closely inbred strain. If, for example, a mouse of strain X be inoculated while still *in utero* with a suspension of cells of emulsified liver, kidney, or spleen from a mouse of strain Y, it will thereafter accept and permanently retain a skin graft from a strain Y animal: the process called by Billingham and his collaborators actively acquired tolerance. These and similar procedures depend for their success upon the fact that embryonic tissues do not have the ability to form antibodies towards the inoculated foreign cells; they cannot yet distinguish between "self" and "not self," and after this maneuver there persists throughout the life of the animal an inability to recognize the Y proteins as foreign. In the words of these authors, "Every degree of tolerance is possible, from that which allows a homograft to live only a few days beyond its normal median expectation of survival to that in which it is permanently accepted by and incorporated into its host."

However these procedures, although they show that a homograft may be endowed with a life as long as that of its host, afford no ready explanation for the relatively long life enjoyed by trophoblast. In pregnancy the tolerance is obviously, as it were, the other way round: the mother's tissues cannot be lulled by a masked potion into acceptance in advance of the tissue antigen in trophoblast in the same way as can the tissues of the mouse inoculated *in utero* with cells from its future donor. Nevertheless, it seems not inappropriate to regard the 40-week survival of trophoblast as a form of prolonged homograft tolerance, and its eventual decay as a prolonged homograft reaction. The viable appearance of some placental polyps suggests that the potential length of survival of trophoblast is longer than 40 weeks, and that in parturition trophoblast meets a premature and accidental death. However, the manifestly degenerative changes within it at term show that its natural death would not have been delayed long. Tolerance of the trophoblastic graft is not complete.

The question then arises whether trophoblast perhaps owes its survival to 1 of 3 things: (1) the existence of some state of complacency or nonreactivity peculiar to tissues of Müllerian duct origin; (2) the fact that it is an offspring-to-parent graft or syngenesiotransplant; and (3) the fact that it is antigenically immature.

The first possibility, the existence of a complacency peculiar to Müllerian duct tissues, is excluded by nature's own transplantation experiment, namely, placentation within the peritoneal cavity in an abdominal pregnancy. As has been reported many times, a placenta implanted wholly within the peritoneum can adequately sustain the needs of a fetus and allow its delivery alive at term. This acceptance of trophoblast by the peritoneum could perhaps be explained on the grounds of the close developmental kinship between the peritoneum and Müllerian duct tissues. Such an explanation, however, is unlikely to be correct in view of the virtual absence of inflammatory reaction around particles of trophoblast that have been carried to and

become impacted within the pulmonary capillaries during pregnancy in the human. A similar absence of reaction has been observed following the intravenous injection of suspensions of autologous trophoblast in mice (Park, 1958). It would thus appear necessary to seek the explanation for the tolerance of trophoblast rather in the trophoblast itself than in its environment.

It is hardly possible to dissociate directly the two other factors that may be concerned in promoting the long survival of trophoblast: its character as a syngenesiotransplant and its possible antigenic immaturity. Indirect evidence, however, suggests that antigenic immaturity is the more important. Syngenesiotransplants of adult tissues tend on the whole to be tolerated better than ordinary homografts (Loeb, 1945), but even so their survival is rarely, if ever, equals that of trophoblast. Embryonic tissue grafts, on the other hand, have frequently been shown to survive longer than grafts of adult tissues. For example, Greene (1943) has shown that early embryonic tissues will survive even heterologous transfer, while Gaillard (1953) has transplanted full-thickness embryonic skin on to burns and found that some of it persists for 6 to 7 months, both observations suggesting strongly that embryonic tissue is to a great extent antigenically immature or inefficient. Further evidence in this connection is afforded by experiments in which part of an embryo is transplanted into its own mother, the situation most closely analogous to that of gestational trophoblast. Reports of experiments of this kind are still comparatively few and have given conflicting results, due in part to species differences. Thus, Rous (1910), using mice, found that tissue survived better in embryo-to-mother grafts than in embryo-to-stranger grafts, while Freund (1911), using rats, found no significant difference. Further experiments of this kind in rats and rabbits have been carried out recently by Woodruff (1958), who transplanted fetal limbs intramuscularly into the respective mothers. Histological examination of the implants removed 14 days later from the still pregnant or recently delivered mothers showed leukocytic infiltration characteristic of a homograft reaction, an observation suggesting that, in these species at any rate, tissues of the embryo proper do possess antigenic competence. However, since grafts from the fetus to the mother were beginning to show evidence of a reaction by the maternal tissues only at the stage when homografts of adult skin to nonpregnant hosts would have been completely destroyed, the competence or antigenicity is probably not of a very high order. Woodruff's experiments were in fact designed primarily as an investigation into the problem why the fetus itself, *qua* homograft, enjoys a freedom from damage not possessed by the ordinary homograft, and from them he concludes that the most probable explanation lies in the anatomical separation of the fetus from its mother; maternal cells, which carry antibodies and are thus potentially harmful, are denied access to the fetus. This is probably the true explanation, but it cannot apply to a trophoblast that is at all times exposed to maternal cells (the leukocytes) and that, particularly in cases of placenta accreta, may have a most intimate contact with the maternal tissues. The possibility remains, therefore, that the cells of the embryo proper and those of its trophoblast, although identical genetically, are not identical antigenically. There

has evidently been some modification or partial loss of immunological identity, and, very possibly, it is to this that trophoblast owes its survival.

The immunological implications of an antigenic modification or loss of this kind, if indeed this be the factor that enables gestational trophoblasts to survive for so relatively long, are interesting in that they recall the immunological concept of cancer proposed originally by Green (1954) and expanded by him in several later articles (for example, Green, 1957). One of the fundamental postulates in this theory is that neoplastic cells lack an identity protein complex of the type that confers on normal cells their individuality, and that it is this loss that constitutes in itself the neoplastic state. By losing the protein complex the cell becomes antigenically neutral and thus indifferent to the normal immunologically regulated processes of cell control and cell disposal. As just mentioned, it seems possible that gestational trophoblast, although a normal tissue, has already gone some way along this path of increasing antigenic deficiency; the trophoblast of a hydatidiform mole may have gone still further; while that of choriocarcinoma may represent journey's end. At any rate, there is no doubting the facts that immunological factors are intimately concerned in tissue transplantation; that trophoblast is a transplant of peculiarly intimate type; and that choriocarcinoma is its malignant expression. In this form of cancer, at least, there is an immunological background already in being; further probing in this direction may well prove rewarding.

References

- BILLINGHAM, R. E., L. BRENT & P. B. MEDAWAR. 1956. Quantitative studies on tissue transplantation immunity III. Actively acquired tolerance. *Phil. Trans.* **B239**: 355-424.
- DONIACH, I., J. H. CROOKSTON & T. I. COPE. 1958. Attempted treatment of a patient with chorioncarcinoma by immunization with her husband's cells. *J. Obstet. Gynaecol. Brit. Empire*. **65**: 553-556.
- EDITORIAL ANNOTATION. 1958. *Lancet*. **2**: 682.
- FREUND, P. 1911. Über experimentelle Erzeugung teratoider Tumoren bei der weissen Ratte. *Beitr. pathol. Anat. u. allgem. Pathol.* **51**: 490.
- GAILLARD, P. J. 1953. *In* Discussion. The homologous transplantation of tissues: clinical aspects. *J. Natl. Cancer Inst.* **14**: 683.
- GREEN, H. N. 1954. An immunological concept of cancer: a preliminary report. *Br. Med. J.* **2**: 1374-1380.
- GREEN, H. N. 1957. The absence of immunological identity in neoplastic cells. *Ann. N. Y. Acad. Sci.* **68**(2): 268-301.
- GREENE, H. S. N. 1943. The heterologous transplantation of embryonic mammalian tissues. *Cancer Research*. **3**: 809-822.
- LOEB, L. 1945. *The Biological Basis of Individuality*. Thomas. Springfield, Ill.
- PARK, W. W. 1958. Experimental trophoblastic embolism of the lungs. *J. Pathol. Bacteriol.* **75**: 257.
- ROUS, P. 1910. Comparison of transplanted tumor and of embryonal tissue transplantation in mouse. *J. Exptl. Med.* **12**: 344-366.
- WOODRUFF, M. F. A. 1958. Transplantation immunity and the immunological problem of pregnancy. *Proc. Roy. Soc.* **B148**: 68-75.

Discussion of the Paper

R. W. NOYES: W. W. Park has stressed the variability in malignancy of the trophoblast and believes that this is an inherent characteristic of the

destructive moles and chorioepitheliomas from their inception. On the other hand, we have heard from other speakers that patients are able to survive for long periods of time in a state of symbiosis with their tumors before succumbing to a rapid terminal invasiveness of chorioepithelioma. I wonder if we have enough evidence at the present time to distinguish between the malignant proclivities of the trophoblast on the one hand and the defensive mechanism of the maternal organism on the other. How does Park explain the fact that some patients may live several years with obvious metastatic chorioepithelioma and yet die so suddenly if the chorioepithelioma has been acutely malignant from the outset?

W. W. PARK: Patients sometimes manage to survive surprisingly long in symbiosis with metastatically malignant neoplasms of various types. Those who do so with metastatically malignant trophoblast are extremely few but the underlying principle is the same: a balance is held for a shorter or longer time between the aggressiveness of the neoplasm and the defensive powers of the host. I do not think we have any more knowledge of the factors that tip the scales, one way or the other, in the case of malignant trophoblast than in the case of any other malignant tissue. All we can do meanwhile, it seems to me, is to interpret the facts as indicating that trophoblast has a wide range of biological behavior; the more aggressively malignant the trophoblast, the sooner will the patient die.

R. L. EHRLMANN (*Boston Lying-In Hospital, Boston, Mass.*): Has anyone ever been successful in transplanting trophoblast from one animal into another?

W. W. PARK: To my knowledge there has been no success in this type of experiment. I attempted to transplant minced trophoblast back into the same mouse without success.

D. ROTH (*New York University-Bellevue Medical Center, New York, N. Y.*): I should like to make two points: one in the form of a question, the other as a plea. The question is: In reference to Park's observation of the absence of inflammatory reaction to trophoblastic tissue transported to the lungs, is this not be a reflection upon its generally intravascular localization, in which site it is relatively safe from the immune reactions of the tissues, in much the same manner as when homologous blood cells introduced by compatible blood transfusion are protected from destruction as would occur were they to escape into the perivascular tissues?

The plea is that we preserve an open mind as to the problem of evaluating the benignancy or malignancy of trophoblastic tumors. Tissues and organs that are so dependent on trophic stimulation from the pituitary as well as from other trophic influences possibly may undergo uncontrolled growth or regression as a result of variations in the extrinsic trophic stimuli or variations in their dependency upon such stimuli rather than as a function of any intrinsic quality of their own component cells. Thus, the determining factor concerned with their potential aggressive behavior may not be disclosed by simple observation of their morphologic characteristics.

W. W. PARK: The necessarily intravascular location of embolic trophoblast indeed could explain the absence of reaction in adjacent tissue, at any rate

for a few days, but I have seen embolic trophoblast in the lungs of patient dying as late as 16 days after parturition and there was still no sign of organization such as would be seen with, for example, embolic thrombus from a peripheral vein. I do agree with D. Roth's plea. Almost certainly we shall need evidence other than the purely morphologic for further understanding of the peculiarities of trophoblast.

QUESTION FROM THE FLOOR: In reference to pulmonary arteritis occurring in association with embolic choriocarcinoma, I should like an explanation of the difference between his inflammatory reaction as cited by W. B. Ober and the absence of reaction around trophoblastic emboli as shown by W. W. Park.

W. W. PARK: The acute arteritis in the case shown by W. B. Ober certainly stands in striking contrast to the virtual absence of reaction around the embolic trophoblast demonstrated in my paper. In Ober's case the tissue was malignant trophoblast, for the patient died from metastasizing choriocarcinoma, and it lay within relatively large pulmonary arteries; in my case the tissue was either normal trophoblast or trophoblast from hydatidiform moles lying within pulmonary arterioles or capillaries. The circumstances are therefore different, but I doubt whether this would really account, to any significant extent, for the difference in tissue responses, for in examples of intrapulmonary choriocarcinoma I have seen I cannot recall any acute arteritis. A reaction of this kind to occlusion of a vessel by neoplastic tissue is unusual at any time, and I feel that one would need to know considerably more about this particular case before attempting to explain the findings so full.

CHORIOCARCINOMA OF THE TESTIS AND EXTRAGENITAL CHORIOCARCINOMA IN MEN

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Choriocarcinoma of the testis presents a striking clinicopathological picture. The fact that the primary growth is frequently small and sometimes occult, the appearance of rapidly fatal hemorrhagic metastases and the endocrinological manifestations secondary to the production of hormones by the tumor have all attracted much attention. The incidence of so-called pure choriocarcinoma in testicular new growths is generally given as between 0.5 and 1 per cent, but this figure has little meaning because multiple sectioning of many apparently uniformly choriomatous growths will reveal the presence of teratomatous or germinomatous elements. Furthermore, no sharp dividing line can be drawn between the morphologically classic choriocarcinoma and many embryonal carcinomas. Finally, foci of choriocarcinomatous tissue are often encountered in primary testicular tumors of other types and even more commonly in their metastases.

Although in the series of testicular tumors reviewed by Friedman and Moore¹ less than 0.4 per cent of the primary neoplasms were pure choriocarcinomas, foci of choriomatous tissue were evident in 6 per cent of the embryonal carcinomas and in 15 per cent of the teratocarcinomas. Dixon and Moore² reported that 25 to 30 per cent of the metastases of testicular teratomas and teratocarcinomas showed choriocarcinoma and that when a focus of chorioma was noted in the primary tumor the metastases were generally choriomatous.

Scully and Parham³ described syncytiotrophoblast in 11 of 16 teratocarcinomas. Marchetti and Lewis⁴ recorded the fact that 57 per cent of primary testicular tumors in their series contained chorionic tissue and that chorioepitheliomas and cytotrophoblastomas made up 16 per cent of the neoplasms. Conversely, other workers have cited an incidence as low as 5 per cent.⁵ Obviously, the criteria employed for the identification of neoplastic trophoblast are not uniform.

Choriocarcinoma of the testis cannot be considered a static entity in the same sense as is epidermoid carcinoma of the skin. Trophoblast is one of the many types of evolving and differentiating tissues that emerge in tumors of germinal origin. The trophoblastic pattern may be present almost from the first in the primary tumor or may not be manifest until much later, in metastases. Although such differentiation occurs in other tumors, it is particularly conspicuous in the teratoid growths because they arise from totipotent elements. This phenomenon is most striking in the case of choriocarcinoma because of the florid aspect of neoplastic trophoblast.

It is generally agreed that the embryonal carcinoma is the precursor of both the trophoblastic and the teratoid tumors. The place that the germin-

oma occupies in the evolutionary scheme of the teratoid tumors has not been established. Many workers have expressed the belief that the germinoma, or seminoma, evolves from the precursor totipotent element and is not the direct ancestor of the embryonal carcinoma. To me, the over-all evidence suggests that the germinoma is a tumor of the primordial extragonadal germ cells and the precursor of the embryonal carcinoma. Perhaps pertinent is the observation that trophoblastic elements occasionally emerge directly from germinomatous cells (FIGURE 1).

The direction of differentiation from germinoma and embryonal carcinoma to chorioepithelioma or teratoma must be borne in mind in order to understand the significance of mixed growths. For example, it is inaccurate to



FIGURE 1. Formation of syncytiotrophoblast in a germinoma of the testis. $\times 95$.

state that a choriocarcinoma develops from a teratoma. Simultaneous or sequential trophoblastic and teratoid differentiation of an embryonal carcinoma leads to the coexistence of a teratoma and a chorioepithelioma. Similarly, the presence of germinomatous tissue adjacent to a choriocarcinoma in the testis can be accounted for by the multicentric origin of germinomas and the failure of certain portions of the tumor to develop beyond the primordial stage. The germinomatous component is sometimes restricted to a few intratubular neoplastic elements which are easily overlooked. The alternative viewpoint, which proposes that the presence of an embryonal carcinoma, for example, induces the formation of germinomatous cells in the adjacent portions of the testis is not consistent with the over-all picture.

Embryonal carcinoma and classic choriocarcinoma represent the two ends of a spectrum; the degree to which intermediate tumors incline toward chorioepithelioma varies widely. In certain embryonal carcinomas the individual cells appear to be cytotrophoblastic. Enlargement of the nuclei and increased basophilia of the cytoplasm are evident, and the cells assumed

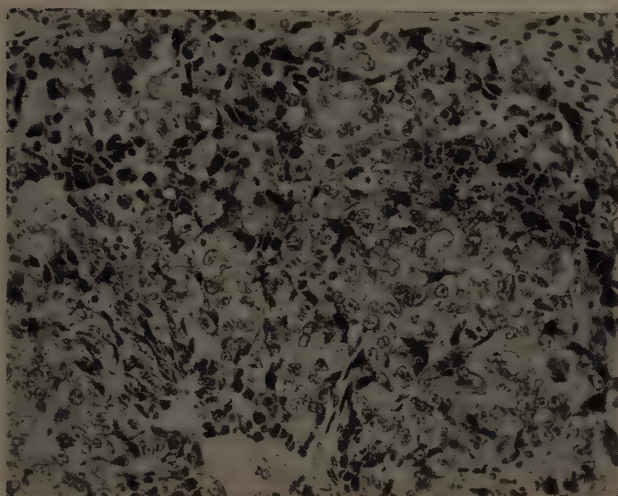


FIGURE 2. Trophoblastic embryonal carcinoma of the testis. The dark cells are the earliest manifestation of syncytiotrophoblastic differentiation. $\times 129$.



FIGURE 3. Syncytiotrophoblast formation about nests of embryonal carcinomatous elements in testicular tumor. $\times 163$.

glandular, papillary, or solid conformations. Cytotrophoblast may appear in a pure form or with a sprinkling of the dark distorted "applied" elements that give the first indication of syncytiotrophoblastic differentiation (FIGURE 2). Syncytiotrophoblast sometimes seems to appear without cytotrophoblast (FIGURE 3). The pattern of the classic choriocarcinoma, in which syncytiotrophoblast envelops masses of cytotrophoblast, is well known. I have never seen mature or molar villi in such neoplasms, but teratoid



FIGURE 4. Pseudovillous structures in spermatic veins draining teratocarcinoma of testis. $\times 65$.

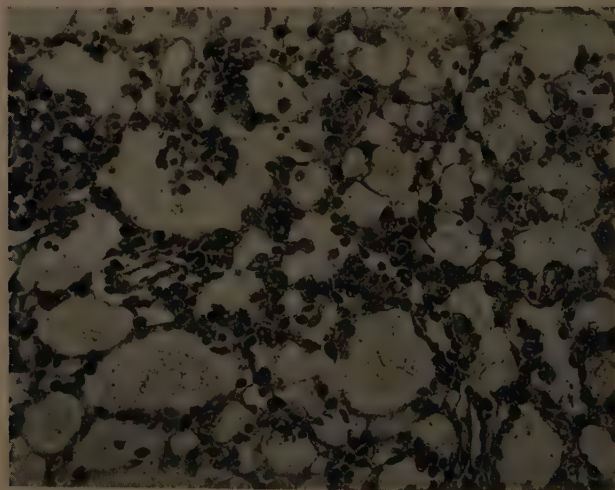


FIGURE 5. Reticular pattern in part of an otherwise classic choriocarcinoma of the testis. The patient had gynecomastia. Reproduced by permission from Jack Gordon, Los Angeles, Calif. $\times 112$.

intravascular growth can simulate molar tissue (FIGURE 4). The reticulated pattern of certain embryonal carcinomatous variants deserves special mention (FIGURE 5); the epithelium forms anastomosing strands, in which the cells are at times epithelioid and at others spindle and stellate. In the meshes of coagulated protein and fibrin or edematous ground substance and mesenchyme may be observed. I believe that such embryonal carcinomas are also trophoblastic.

Not all embryonal carcinomas are pretrophoblastic; many somatic tissues emerge from the epithelial complexes to produce teratocarcinomas and teratomas. Precursors of many epithelial structures are present, although neural and entodermal differentiation are manifest most often. Of special interest are the tiny embryoid bodies,⁶ many of which may be present in a single tumor. These structures mimic the appearance of early embryos and often have a trophoblastic component. Embryoid bodies are encountered in ovarian as well as in testicular neoplasms. Since embryonal carcinomas obviously give rise to neoplastic trophoblast as well as to neoplastic somatic tissue, a designation such as germinal carcinoma might be more appropriate than embryonal carcinoma. Teilum⁷ suggested mesoblastoma as a suitable

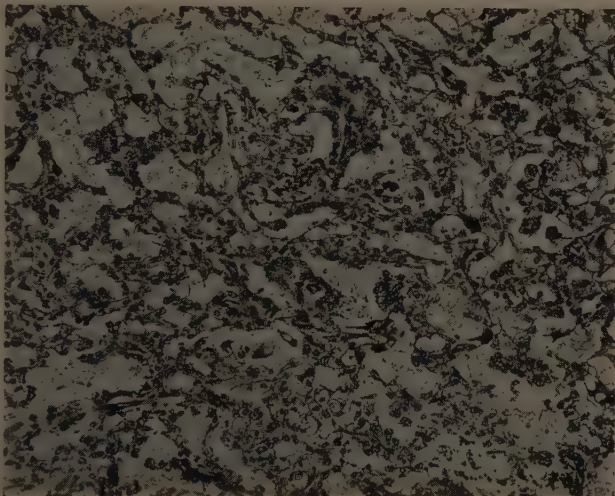


FIGURE 6. Reticular embryonal carcinoma of the ovary in a 39-year-old woman. Tests for chorionic gonadotrophin positive. $\times 65$.

designation for the precursor of the trophoblastic tumors and reserved embryonal carcinoma as a term for the presomatic growths.

Teilum also emphasized the existence of a form intermediate between gonocytoma (germinoma) and choriocarcinoma, a tumor which can produce chorionic gonadotrophin in the absence of frankly chorionomatous tissue. He pointed out that the tumor that I call the reticular form of embryonal carcinoma has often been incorrectly classified as mesonephroma. This neoplasm has also been considered to be a mesothelioma or even a malignant adenomatoid tumor. Teilum's clarification and homologization of the ovarian and testicular tumors of the germinoma-embryonal carcinoma-chorioepithelioma series have been most useful. The relative frequency with which adult teratomas develop in ovarian germinal tumors has obscured the fact that embryonal carcinomas and choriocarcinomas per se occur in the female gonad. They appear most frequently before puberty, occasionally after the menopause and rarely during the reproductive period (FIGURES 6, 7, 8).

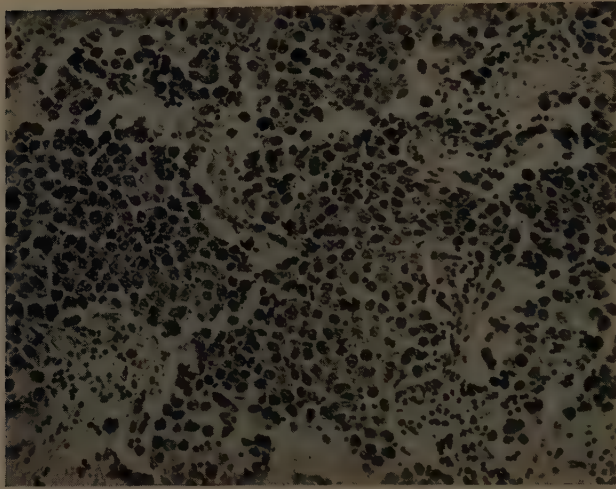


FIGURE 7. Germinomatous portion of ovarian tumor in 9-year-old girl. Reproduced by permission from I. Tilden, Honolulu, Hawaii. $\times 112$.

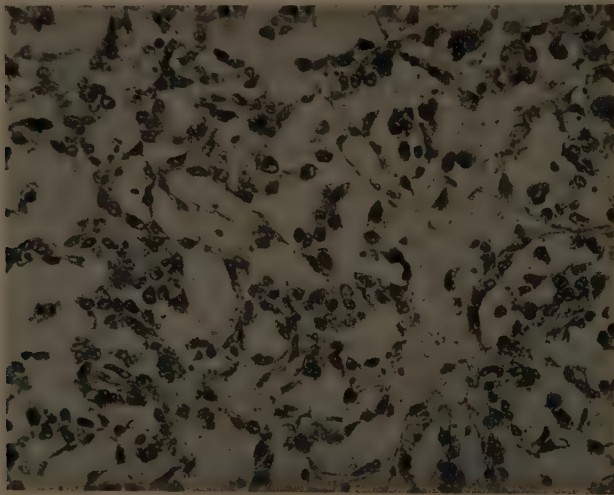


FIGURE 8. Reticular embryonal carcinomatous portion of same tumor as in FIGURE 10. $\times 112$.

Choriocarcinoma of the testis usually occurs in young adults. It is not clear whether the carcinomas encountered in boys before puberty⁸ are pre-trophoblastic embryonal carcinomas, and the occurrence of germinomas has not been described in patients of this age group. Choriocarcinoma has been encountered in the testis of a newborn boy,⁹ but the possibility of metastasis from the mother was not ruled out. Rarely, a cryptorchid testis may be the seat of such a growth, although the tumor characteristic of the undescended gonad is germinoma.

Invasion of vessels and production of hormones by trophoblast and cytological alterations of the pituitary are normally seen in pregnant women as well as in men with choriocarcinomas. Such production of hormones is responsible for the appearance of gynecomastia, testicular tubular atrophy and hyperplasia of interstitial cells (FIGURE 9). The invasion of blood vessels results in widespread hematogenous metastases and hemorrhagic necrosis and may even lead to extinction of the tumor. I have also seen extensive necrosis in the hypophysis identical with that seen in *post partum* lesions of the pituitary. The occurrence of necrosis under such circumstances casts doubt on the conventional explanation that this reaction in the postgestational woman results from *post partum* hemorrhage and shock.

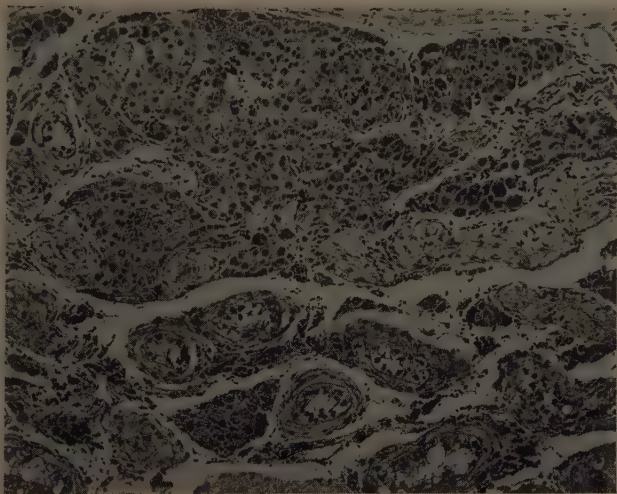


FIGURE 9. Tubular atrophy and interstitial hyperplasia in testis containing trophoblastic embryonal carcinoma. $\times 65$.

I have also observed another unusual hormonal reaction, edema and hyperplasia of a testicular hydatid in a patient with a primary chorionepithelioma of the mediastinum; the hydatid was so enlarged that the prosector deemed it a primary teratoma.

The most striking manifestation of the local effects of chorionic gonadotrophin that I have encountered was in a young man with a germinomatous, embryonal carcinomatous, and choriocarcinomatous neoplasm of the testis. Adjacent to nests of trophoblastic epithelium were clusters of Leydig cells, which were obviously not interstitial cells in residual testicular parenchyma, but elements differentiating from teratoid stroma (FIGURE 10).

Endocrinological manifestations support the concept of the interrelation between choriocarcinoma and embryonal carcinoma. The presence of chorionic gonadotrophin and its effects have been demonstrated in patients with pure embryonal carcinoma.¹⁰ Friedman and Moore¹ pointed out that as many patients with embryonal carcinoma or teratocarcinoma had gynec-

comastia as did those with choriocarcinoma. Recently, Pierce and his co-workers¹¹ have assembled experimental data that substantiate this viewpoint. An embryonal carcinoma that produced chorionic gonadotrophin in a human patient continued to grow as an embryonal carcinoma and to produce gonadotrophin when transplanted into hamsters. The results of this experiment are consistent with the view that cytotrophoblast rather than syncytium produces gonadotrophin.

According to the Dixon-Moore² survey 30 per cent of the patients with embryonal carcinoma had positive reactions to the tests for gonadotrophin, although chorionic gonadotrophin was not specifically assayed. The presence of gonadotrophin was associated with a poor prognosis. Moon and

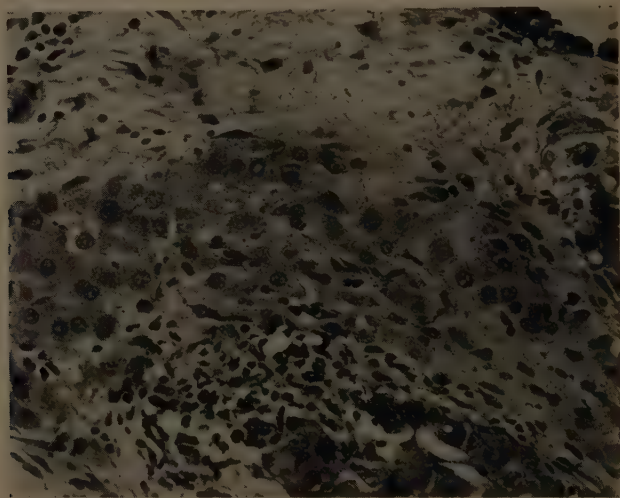


FIGURE 10. Formation of Leydig cells in teratoid stroma adjacent to nests of trophoblastic embryonal carcinoma in a testicular tumor. Reproduced by permission from Robert A. Blossom, Whittier, Calif. $\times 190$.

Hullinghorst¹² did not even attempt to separate choriocarcinoma from embryonal carcinoma because, as they pointed out, hormonal activity, hyperplasia of interstitial cells, and mortality rates are virtually identical for the two tumors. They emphasized the tendency for these histological types to merge and expressed the opinion that the two neoplasms are histogenetically identical.

The mortality rate for patients with pure choriocarcinoma of the testis approaches 100 per cent, and the patient often dies within two years. I studied one young man with choriocarcinoma who died only a few days after the onset of what appeared to be, clinically, hemorrhagic pneumonitis. This tumor metastasizes widely and characteristically invades the lung, liver, and brain; regional and distant lymph nodes are frequently involved. Metastasis to locations unusual for ordinary carcinomatous spread are the rule.

The spectacular nature of choriocarcinoma is matched by the range of bizarre theories of its origin. One speculation¹³ is that all carcinomas of somatic tissues are actually trophoblastic in origin; another theory is that trophoblast derives from granulosa.¹⁴ A third idea that has been expressed is that trophoblast stems from the male gamete.¹⁵ It seems likely, however, that the tendency for neoplastic germinal tissue to form adult teratomas in the ovary and to appear as embryonal carcinomas and choriocarcinomas in the testis results from differing environmental influences and endocrine constellations acting on comparable genetic materials. It is interesting to note that in only about one half of the choriocarcinomas and teratomas in men,¹⁶⁻¹⁸ but in virtually all of the extrauterine growths in women, female sex chromatin is discernible. The suggestion that choriocarcinoma results from autofertilization¹⁹ makes necessary the assumption that gametes of the opposite sex are present in the gonad. The most reasonable viewpoint appears to be that choriocarcinoma evolves from neoplastic germinal elements, possibly derived from primordial germ cells. Parthenogenesis need not be invoked, since carcinomas of many tissues, once neoplastic change has set in, can express the multiple potentialities of the homologous embryonal element.

Spectacularly, either the primary tumor or a metastasis of a choriocarcinoma of the testis may regress.^{20, 21} Whether regression ensues from infarction resulting from angioinvasiveness of the tumor or from senescence of trophoblastic tissue after a relatively brief life is debatable. Other factors may be involved. A complementary manifestation is the tendency toward somatic differentiation and maturation, which may become manifest in a primary growth and result in the formation of an adult teratoma after the tumor has metastasized as a choriocarcinoma. Regression following irradiation has also been observed. Syncytiotrophoblast is relatively radio-resistant; however, a rare embryonal carcinoma may prove to be somewhat radioresponsive.

Although choriocarcinoma in men arises in a variety of sites other than the testis, there is a tendency for the tumor to localize in specific organs and tissues. This specificity of localization must stem from unknown embryological factors, because there is nothing obvious in the structure or function of the organs or tissues in which these growths appear that would explain the incidence of the neoplasm. Extragenital choriocarcinomas have been described in the retroperitoneum, in the mediastinum, and in the pineal (FIGURE 11). The rare occurrence of this tumor in such organs as the lung, liver, stomach, bladder, and kidney has been reported.

The appearance of teratomas, teratocarcinomas, and embryonal carcinomas has been reported a number of times in the heart and thyroid and in the cervical, sacrococcygeal, and retroperitoneal regions. Frequently the embryonal carcinomatous component of a teratoma has gone unrecognized, and the tumor has been characterized as a malignant teratoma. Trophoblast was actually recognizable in one of Bale's²² teratomas of the thyroid. It must be reemphasized that adult teratomatous tissue rarely becomes malignant and that the carcinomatous structures seen in teratomas are not

the descendants but the ancestors of the adult tissues. Exceptions to this rule are the teratoid tumors of the ovary, in which an epidermoid carcinoma, for example, may arise from teratomatous squamous epithelium.

The concept that choriocarcinoma of the pineal occurs almost entirely in boys is underlined by the facts that in one review²³ 75 of 81 teratoid growths occurred in males and that most of the patients were under 20 years of age. Doubtless the involvement of the hypothalamus and the production of hormones by the neoplastic tissue are jointly responsible for the endocrine effects, which include precocious puberty,²⁴ polyglandular insufficiency, and diabetes insipidus.

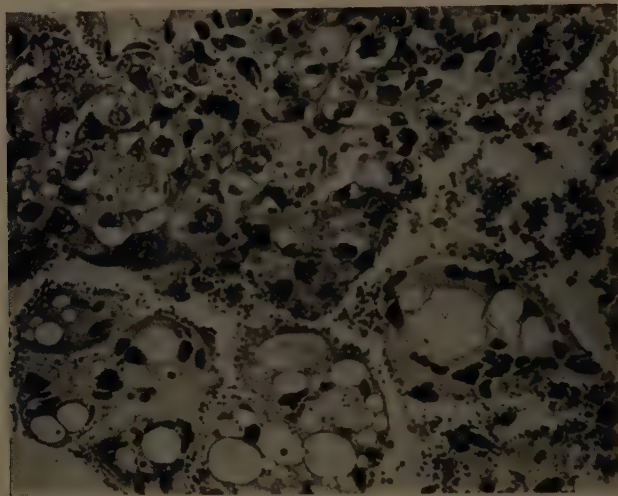


FIGURE 11. Choriocarcinoma of the pineal. Reproduced by permission from Norman Elton, Johnson City, N. Y. $\times 122$.

Chorioma of the pineal often arises in conjunction with teratoma, embryonal carcinoma, and/or germinoma.^{25, 26} The fact that the same four types of tumor occur singly or in conjunction in both the pineal gland and the testis suggests that they must have a common origin.

Tumor often destroys the pineal gland, but calcific concretions mark its former position. It has been established that some pinealomas arise in such extraneous locations as the third ventricle, the infundibulum, and the neurohypophysis.²⁷ It might be wise, therefore, to designate the pineal germinomas and teratoid tumors as diencephalic. These tumors bear little or no relation to normal pineal tissue or to the neurogenic pinealoma. The fact that embryonal carcinomas and choriocarcinomas of the pineal metastasize to extracranial organs^{28, 29} emphasizes the basic differences between these tumors and other neoplasms of the brain.

Mediastinal chorionepithelioma (FIGURE 12) usually occurs in young male patients,³⁰ but an occasional tumor in a female has been recorded.³¹ Ordinarily the boy is past puberty before the tumor appears but, in a few instances,

I have examined patients with the tumor who were prepuberal. Willis³² has stated that the incidence of mediastinal teratomas is the same in males and females, but that the malignant neoplasms appear preponderantly in male patients. The fact that embryonal, teratoid, and trophoblastic growths of the mediastinum are often found in association with germinomas^{34, 35} makes it difficult to accept Schlumberger's concept³³ that these tumors are of somatic rather than germinal origin. Schlumberger has demonstrated that the teratoids of the mediastinum arise specifically in the thymus and not in the mediastinum.³⁴⁻³⁵ The tuberculoid granulomas evident in the gonadal and pineal germinomas are also apparent in the mediastinal germinomas.

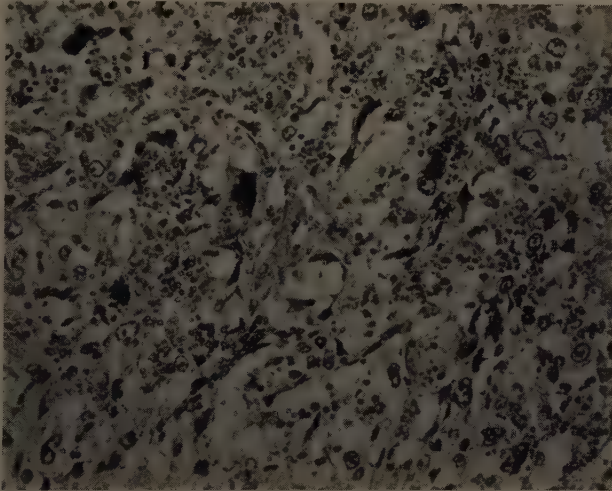


FIGURE 12. Choriocarcinoma of the thymus in a thirty-seven-year old man. $\times 122$.

Solomon³⁶ reported a metastasizing cardiac teratoma in a two-year-old girl. The third illustration in his paper shows an embryonal carcinoma of the reticular type. This cardiac neoplasm could have arisen from overlying or included thymic tissue. Aberrant thymic tissue in the neck and about the thyroid may be the site of origin of some of the cervicothyroidal teratoid growths.²² Some choriomas that appear to have originated in the lung³⁷ may actually have evolved from the thymus.

The testis as the primary site must be excluded before a diagnosis of primary extragenital choriocarcinoma can be made. Both testes must be studied either by multiple or serial section, and the absence of tumor, cicatrix, or intratubular germinomatous elements must be established.^{38, 39}

Theoretically, choriocarcinoma can arise in the retroperitoneum, because the urogenital ridge extends well cephalad of the level that is usually involved, and actual testicular tubules have been described in aberrant retroperitoneal gonadal tissue. However, in no case of retroperitoneal choriocarcinoma or embryonal carcinoma in which it was possible to examine the entire testis have I failed to find a tumor or a cicatrix.²⁰ The rare renal chorioma can be

considered either as a variant of the retroperitoneal type or as one of the growths that occur in parenchymal organs.

I have seen trophoblastic embryonal carcinoma in sacrococcygeal teratoid tumors, but have never encountered frank choriocarcinoma or germinoma. The sacrococcygeal teratomas are often exceptionally well differentiated and display a variety of entodermal and neural derivatives. Proliferating neuro-epithelium sometimes simulates embryonal carcinoma. It is important to emphasize the fact that the sacrococcygeal teratoids appear almost twice as often in females as in male patients.^{40, 41} It is not clear whether the neoplasms in the reports by Levine *et al.*⁴² and Rhoden⁴³ bear any relation to the tumors under discussion here. In each instance the tumor was a sacrococcygeal teratoma; in one case pituitary gonadotrophin (FSH) was identified and, in the other, a diffuse proliferation of adrenogenital elements reminiscent of the formation of Leydig cells in a trophoblastic tumor (FIGURE 10) was described.

Choriocarcinomas arise rarely in such epithelial organs as the liver, stomach, or bladder, sites in which neither teratomas nor germinomas have been commonly observed. Willis³² and others⁵ have expressed doubt about the trophoblastic nature of such choriocarcinomas and certain embryonal carcinomas because giant cells and necrosis are not infrequently evident in carcinomas of somatic origin. Although some so-called choriocarcinomas reported in the literature would not withstand rigorous scrutiny, I feel that Willis has been overly critical.

Some growths are purely choriomatous and others exhibit transitions from neoplasms characteristic of the involved organ. Occasionally metastases from a somatic carcinoma appear trophoblastic. Voss⁴⁴ recently reported a choriocarcinoma of the stomach associated with a polypoid carcinoma; there were metastases in the liver and lungs, but the mediastinum and retroperitoneum were free, and the testes showed neither scar nor tumor. The Aschheim-Zondek test was positive, and the patient had gynecomastia. Other tumors that have been reported have not been so completely studied. For example, Hartz and Ramirez⁴⁵ published a case in which there was involvement of the stomach by both mucinous carcinoma and choriocarcinoma. Hyman and Leiter⁴⁶ described a vesical chorioepithelioma.

One can theorize that the extragenital choriocarcinomas arise from the primordial germ cells, which are widely disseminated throughout the embryo before they ultimately reach the developing gonad. This theory is supported by the occurrence of germinomas in the diencephalic and thymic regions. Witschi⁴⁷ has published photographs of human primordial germ cells at many different sites in the embryo; these elements bear a striking similarity to those encountered in germinomas. If one accepts the concept that the germinoma is a tumor comprised of primordial germ cells, the gonadal and extragenital choriocarcinomas must have a common origin.

References

1. FRIEDMAN, N. B. & R. A. MOORE. 1946. Tumors of the testis: a report on 922 cases. *Military Surg.* **99**: 573-593.

2. DIXON, F. J. & R. A. MOORE. 1951. Tumors of the male sex organs. Atlas of Tumor Pathology. Sect. 8 fascicles 31b. and 32. Armed Forces Inst. Pathol. Washington, D. C.
3. SCULLY, R. E. & A. R. PARHAM. 1948. Testicular tumors: I. Seminoma and teratoma. A. M. A. Arch. Pathol. **45**: 581-607.
4. MARCHETTI, A. A. & L. G. LEWIS. 1952. Homologous ovarian and testicular tumors. Am. J. Obstet. Gynecol. **3**: 294-306.
5. GRAY, C. P., J. R. McDONALD & G. J. THOMPSON. 1950. Chorio-epitheliomatous elements occurring in teratoma of the testis. Am. J. Surg. **79**: 653-659.
6. EVANS, R. W. 1957. Developmental stages of embryo-like bodies in teratoma testis. J. Clin. Pathol. **10**: 31-39.
7. TEILUM, G. 1946. Gonocytoma: homologous ovarian and testicular tumors. I. with discussion of "mesonephroma ovarii." Acta Pathol. Microbiol. Scand. **23**: 242-251.
8. SILVERT, D. 1957. Embryonal carcinoma of testis in infants and children. J. Urol. **78**: 269-277.
9. EMERY, J. L. 1952. Chorionepithelioma in a newborn male child with hyperplasia of the interstitial cells of the testis. J. Pathol. Bacteriol. **64**: 735-739.
10. BREWER, J. I. 1946. Chorionic gonadotropin in the diagnosis of testicular tumors. A. M. A. Arch. Pathol. **41**: 580-591.
11. PIERCE, G. B., E. L. VERNEY & F. J. DIXON. 1958. Endocrine studies on hetero-transplanted human testicular cancer. Proc. Am. Assoc. Cancer Research. **2**: 334.
12. MOON, H. D. & R. L. HULLINGHORST. 1948. Basic patterns in teratoid tumors of the testis. Am. J. Pathol. **24**: 1067-1081.
13. GURCHOT, C., E. T. KREBS, JR. & E. T. KREBS. 1947. Growth of human trophoblast in eye of rabbit: Its relationship to the origin of cancer: A preliminary report. Surg. Gynecol. & Obstet. **84**: 301-312.
14. GORDON, I. 1949. The origin of placental trophoblast and the function of placental gonadotrophin. Lancet. **1**: 807-814.
15. HIRSCH, E. F. 1949. Extragenital choriocarcinoma: with comments on the male origin of trophoblastic tissues. A. M. A. Arch. Pathol. **48**: 516-524.
16. HUNTER, W. F. & B. LENNOX. 1954. The sex of teratomata. Lancet. **2**: 633-634.
17. OOSTERHUIS, W. W. & I. S. LEVIJ. 1957. Geslachtschromatine in chorionepitheliomen en in carcinomen van de huid. Ned. Tijdschr. Geneesk. **101**: 901-903.
18. MANCINI, A. M. 1956. Geschlechtsbestimmung am Zellkern zur Klärung der Pathogenese der Teratome und Hodengeschwulste. Z. Krebsforsch. **61**: 376-380.
19. PETILLO, D. 1944. A critical review of the pathogenesis of chorioma testis and a new theory. Urol. Cut. Rev. **48**: 53-66.
20. FRIEDMAN, N. B. 1951. The comparative morphogenesis of extragenital and gonadal teratoid tumors. Cancer. **4**: 265-276.
21. RATHER, L. J., W. R. GARDINER & J. B. FRERICHs. 1954. Regression and maturation of primary testicular tumors with progressive growth of metastases: a report of six new cases and a review of the literature. Stanford Med. Bull. **12**: 12-25.
22. BALE, G. F. 1950. Teratoma of the neck in the region of the thyroid gland: a review of the literature and report of four cases. Am. J. Pathol. **26**: 565-579.
23. KITAY, J. I. & M. D. ALTSCHULE. 1954. The Pineal Gland: A Review of The Physiologic Literature. Harvard Univ. Press. Cambridge, Mass.
24. ZONDEK, H., A. KAATZ & H. UNGER. 1953. Precocious puberty and chorioepithelioma of the pineal gland; with report of a case. J. Endocrinol. **10**: 12-16.
25. RUSSELL, D. S. 1944. The pinealoma: its relationship to teratoma. J. Pathol. Bacteriol. **56**: 145-150.
26. FRIEDMAN, N. B. 1947. Germinoma of the pineal: its identity with germinoma ("seminoma") of the testis. Cancer Research **7**: 363-368.
27. TROLAND, C. E. & C. A. BROWN. 1948. Precocious puberty of intracranial origin. J. Neurosurg. **5**: 541-555.
28. STOWELL, R. E., E. SACHS & W. O. RUSSELL. 1945. Primary intracranial chorionepithelioma with metastases to the lungs. Am. J. Pathol. **21**: 287-301.

29. TOMPKINS, V. N., W. HAYMAKER & E. H. CAMPBELL. 1950. Metastatic pineal tumors: A clinico-pathologic report of two cases. *J. Neurosurg.* **7**: 159-169.
30. HOUGHTON, J. D. 1936. Malignant teratoma of the mediastinum; report of a case and review of 24 cases from the literature. *Am. J. Pathol.* **12**: 349-372.
31. FANGER, H. & R. MACANDREW. 1952. Extragenital chorionepithelioma in a female arising from a mediastinal teratoma. *Rhode Island Med. J.* **35**: 259-260.
32. WILLIS, R. A. 1951. Teratomas. *Atlas of Tumor Pathology*. Section 3 fascicle 9. Armed Forces Inst. Pathol. Washington, D. C.
33. SCHLUMBERGER, H. G. 1946. Teratoma of the anterior mediastinum in the group of military age: a study of 16 cases and a review of theories of genesis. *A. M. A. Arch. Pathol.* **41**: 398-444.
34. PUGSLEY, W. S. & R. L. CARLETON. 1953. Germinal nature of teratoid tumors of the thymus. *A. M. A. Arch. Pathol.* **56**: 341-347.
35. INADA, K. & A. NAKANO. 1958. Structure and genesis of the mediastinal teratoma. *A. M. A. Arch. Pathol.* **66**: 183-189.
36. SOLOMON, R. D. 1951. Malignant teratoma of the heart: Report of case with necropsy. *A. M. A. Arch. Pathol.* **52**: 561-568.
37. KAY, S. & W. G. REED. 1953. Chorioepithelioma of the lung in a female infant seven months old. *Am. J. Pathol.* **29**: 555-567.
38. LAIPPLY, T. C. & R. A. SHIPLEY. 1945. Extragenital choriocarcinoma in the male. *Am. J. Pathol.* **21**: 921-933.
39. KANTROWITZ, A. R. 1934. Extragenital chorionepithelioma in a male. *Am. J. Pathol.* **10**: 531-544.
40. HICKEY, R. C. & J. M. LAYTON. 1954. Sacrococcygeal teratoma: emphasis on the biological history and early therapy. *Cancer.* **7**: 1031-1043.
41. RAVITCH, M. M. & E. I. SMITH. 1951. Sacrococcygeal teratoma in infants and children. *Surgery.* **30**: 733-762.
42. LEVINE, S. Z., H. L. BARNETT, M. SHIBUYA & J. K. BARBER. 1956. Isosexual precocity in boys including a case of a gonadotrophin-producing teratoma. *Advances Pediatrics.* **8**: 53-95.
43. RHODEN, A. E. 1944. Precocious sexual and somatic development in male infant with presacral teratoma containing androgen-producing tissue, with discussion of mechanism of precocity caused by teratomas. *J. Clin. Endocrinol.* **4**: 185-193.
44. VOSS, C. 1954. Primares chorionepitheliom des magens. *Arch. pathol. Anat. u. Physiol., Virchow's.* **325**: 455-464.
45. HARTZ, P. H. & A. RAMIREZ. 1953. Coexistence of carcinoma and chorioepithelioma in the stomach of a young man. *Cancer.* **6**: 319-326.
46. HYMAN, A. & H. E. LEITER. 1943. Extratesticular chorioepithelioma in a male probably primary in the urinary bladder. *J. Mt. Sinai Hosp.* **10**: 212-219.
47. WITSCHI, E. 1948. Migration of the germ cells of human embryos from the yolk sac to the primitive gonadal folds. *Contribs. Embryol. Carnegie Inst. Wash.* **32**(209): 69-80.

Discussion of the Paper

G. B. PIERCE (*School of Medicine, University of Pittsburgh, Pittsburgh, Pa.*): N. B. Friedman has done remarkable work in showing the morphologic relationships between ovarian and testicular tumors, as well as the relationship of teratoid tumors of the testis to those of the mediastinum and pineal. My interest in germinal tumors of the testis concerns their pathogenesis, specifically the position of embryonal carcinoma in relation to the development of the well-differentiated somatic and trophoblastic tissues common to teratocarcinomas. I believe, in common with Friedman and many others, that embryonal carcinoma cells are probably multipotential and that under given circumstances, they are capable of originating a variety of either somatic or trophoblastic tissues.

As a result of manipulation of a transplantable teratocarcinoma of the testis of a mouse, in an experiment to be described, my group showed that the somatic structures of that teratocarcinoma arose by morphogenesis of embryonal cells.¹ During the conversion of the teratocarcinoma of the mouse to an ascitic form, small cystic structures were found floating in the hemorrhagic peritoneal fluid of the host (FIGURE 1). The cysts were shown by serial section (over 150 cysts have been serially sectioned) to be composed of only 3 embryonic-appearing cell types. There was an investing layer of visceral yolk sac, beneath which lay a mesenchymal tissue in the capillaries of which were found immature hematopoietic cells. Deeper in the cysts were foci of embryonal carcinoma cells (FIGURE 1*b*). These cell types were often arranged in patterns suggesting stages of early embryological development of the mouse, prior to $8\frac{1}{2}$ days gestation. The cysts, when grafted in the subcutaneous space, proved to be teratogenic, giving rise to as many as 12 well-differentiated somatic tissues. Whether these somatic tissues arose from the embryonal carcinoma of the cyst alone or from one of the other two cell types or from a combination of these remains to be seen. Experiments are now being performed that may answer this question.

As far as trophoblastic tumors are concerned, my co-workers and I have successfully heterografted several of these of human testicular origin to the cheek pouches of cortisone-treated hamsters.^{2, 3} One named Pitt 61 grew and metastasized in the human host as an embryonal carcinoma and was associated clinically with high levels of chorionic gonadotrophin. In 20 per cent of the cortisone-treated hamsters in which it has been grafted, Pitt 61 has produced large amounts of chorionic gonadotrophin (FIGURE 1*c*). Neither choriocarcinoma nor syncytiotrophoblast has been found in any of the more than 3000 transplants of Pitt 61 that have been examined histologically over the past 3 years (FIGURE 1*d*). We feel that this embryonal carcinoma has acquired the function of trophoblast, but not the morphology, and as such may be interpreted as representing a stage in the genesis of choriocarcinoma.³

Two choriocarcinomas of human testicular origin have been heterografted to cortisone-treated hamsters in which they have consistently produced chorionic gonadotrophin. One of these, Pitt 89, has been grown in either estrogen- or testosterone-treated hamsters or in oophorectomized, orchietomized or hypophysectomized hamsters with no alteration in the growth rate of the tumors or hormone secretion under the conditions of the experiments (FIGURE 1*e*). These negative experiments indicate that Pitt 89 is an autonomous tumor apparently independent of external endocrine stimulation.

A series of Pitt-89-bearing animals was treated with methotrexate. A dose of 3 mg./kg. of hamster weight daily for 5 days beginning on the day of grafting with tumor, followed by another course after a rest period of 5 days, resulted in a 40 per cent decrease in the growth rate of the tumor, but no apparent alteration of the gonadotrophin production by the tumor. No sizable tumors, well established in the hamsters, were destroyed as a result of methotrexate therapy.⁴ These results correlate quite well with those of

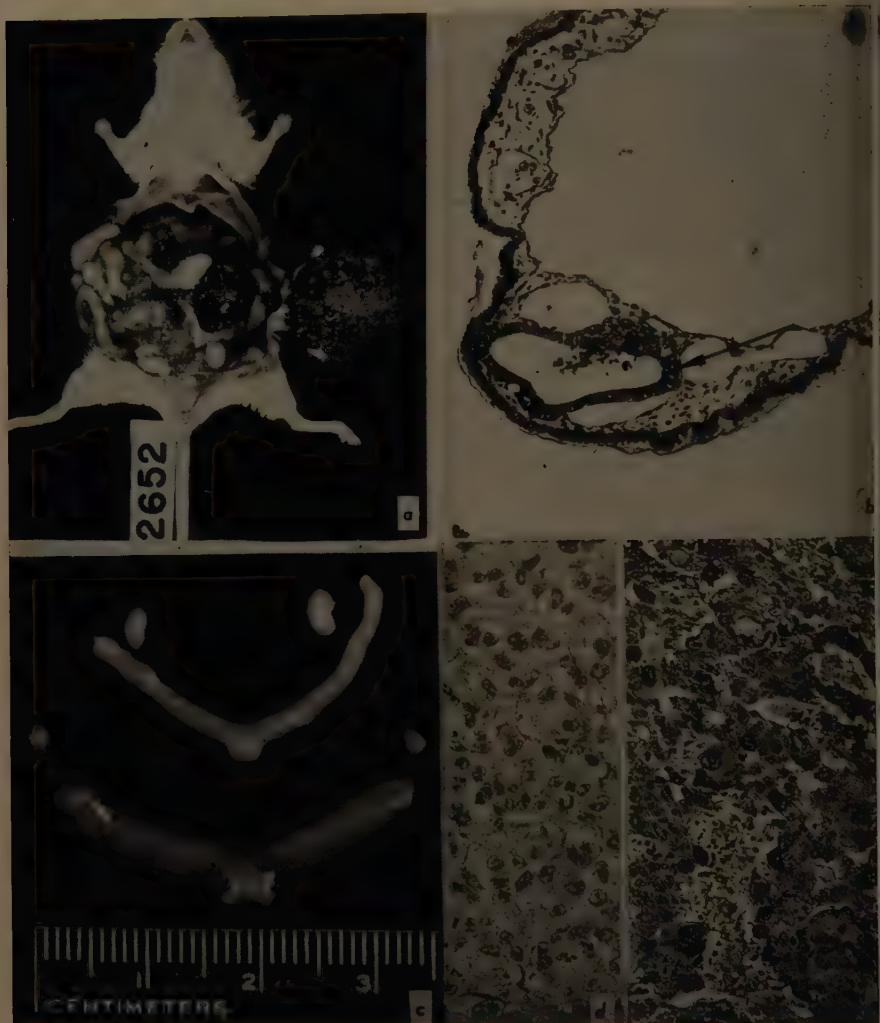


FIGURE 1. (a) First passage of teratocarcinoma intraperitoneally, illustrating solid peritoneal implants and ascitic fluid containing cysts (on the animal's left).

(b) Section of a cyst illustrating investing yolk sac, with underlying capillaries, some of which contain hematopoietic cells. Note the embryonal carcinoma cells embedded in mesenchyme (arrow). $\times 120$.

(c) Uterus weighing 458 mg. from a chorionic gonadotrophin-secreting Pitt 61 (below); control weighing 96 mg. (above).

(d) Typical section of a gonadotrophin-secreting Pitt 61, illustrating typical embryonal carcinoma cells. $\times 240$.

(e) Typical section from Pitt 89, transplantable choriocarcinoma of male origin, illustrating syncytiotrophoblastic giant cells and cytotrophoblast. $\times 120$.

Hertz *et al.* who reported equivocal success in the methotrexate therapy of choriocarcinoma of male origin.⁵

References

1. PIERCE, G. B. & F. J. DIXON. 1958. Testicular teratomas. I. The demonstration of teratogenesis by morphogenesis of multipotential cells. *Cancer*. In press.
2. PIERCE, G. B., E. L. VERNEY & F. J. DIXON. 1957. The biology of testicular cancer. I. Behavior after transplantation. *Cancer Research*. **17**: 134-138.
3. PIERCE, G. B., F. J. DIXON & E. VERNEY. 1959. Endocrine function of a heterotransplantable human embryonal carcinoma. *A. M. A. Arch. Pathol.* **67**: 204-210.
4. VERNEY, E. L., G. B. PIERCE & F. J. DIXON. 1958. Biology of testicular cancer. III. Heterotransplantable choriocarcinoma. *Cancer Research*. In press.
5. LI, MIN CHUI, R. HERTZ & D. B. SPENCER. 1956. Effect of methotrexate therapy upon choriocarcinoma and chorioadenoma. *Proc. Soc. Exptl. Biol. Med.* **93**: 361.

GEOGRAPHIC VARIATION IN THE OCCURRENCE OF HYDATIDIFORM MOLE AND CHORIOCARCINOMA*

*By The Joint Project for Study of Choriocarcinoma and Hydatidiform
Mole in Asia†*

In the past several years there have been numerous reports indicating a higher frequency of trophoblastic tumors in certain Asian countries than in the United States but, because of inadequate communication, it has not been generally known whether these differences are due to varying criteria of diagnosis or whether the higher Asian frequency is real.^{1, 4, 5, 11, 13, 15}

The present study was undertaken in order to document reports concerning trophoblastic tumors in Asian countries and, if possible, to determine some of the factors responsible for their more frequent occurrence. A preliminary study has previously been reported by one of us.⁹

Material and Methods

Asian pathologists and clinicians collaborated in collecting from their files clinical and histological material illustrating all questionable or definite examples of trophoblastic tumors for the years 1948 to 1952. It was decided that one of us should screen the material from each country in order to apply uniform criteria of diagnosis and to assure adequate documentation for statistical studies. In 1953 the pathological material and clinical data were collected by the coordinator of the project and these were assembled at the Armed Forces Institute of Pathology, where histological preparations were cut and studied. Hugh Grady and Arthur Hertig were consulted in the screening of the pathological material, and members of the medical statistics section assisted in the analysis of the data.

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A total of 806 cases of suspected trophoblastic tumors was available for review from Asian countries. An additional 46 examples from Nagasaki, Japan were contributed by the Atomic Bomb Casualty Commission. For comparison with the Asian series, a sample was taken from the files of the Armed Forces Institute of Pathology representing various contributions from United States patients in military and civilian hospitals. Cases with inadequate clinical data or with incomplete tissue were arbitrarily excluded from the study.

The tissue was prepared in the histopathological laboratories of the Armed Forces Institute of Pathology, and the cases were screened according to the following morphologic criteria:

(1) Hydatidiform mole: molar villi had to be clearly distinguished histologically, and gross data indicate bulky quantities of these villi. Many cases of obvious hydatidiform mole were eliminated from the study because of inadequate gross descriptions.

(2) Chorioadenoma destruens: all of the specimens were hysterectomy specimens and villi were distinguished well within the myometrium. Incompatibility of diagnosis was slight in this group, except in three examples of heavy trophoblastic proliferation where villi had not been present in the original sections and the diagnosis of choriocarcinoma was made erroneously.

(3) Choriocarcinoma: this diagnosis was established by the demonstration of trophoblastic tissue invading the uterus, by the presence of metastatic lesions in biopsy or autopsy material, or in abundant curettage specimens in which follow-up information confirmed the diagnosis. All questionable and probable cases were eliminated from the final tabulation. In this group errors of diagnosis occurred most frequently in examples of trophoblastic proliferation at the placental site or at molar sites and in the various bizarre patterns of "syncytial endometritis."

Included in the study from Nagasaki were only the individuals in the control group, that is, those at the time of the atomic bomb explosion beyond 10,000 meters from the hypocenter. Those closer to the hypocenter were eliminated because preliminary statistical tests demonstrated a significant age difference in this "exposed" group.

Among the 647 coded examples of trophoblastic tumors among random contributions to the files of the Armed Forces Institute of Pathology there was a larger number of Asians than is found in samples of other diseases at the same institute. These cases were therefore discarded from the study, as were those with inadequate clinical and follow-up information. Only 195 patients from the United States were selected for analysis; of this group, 90 per cent were white and 10 per cent Negro, a racial distribution closely approaching that of the general population.

Despite the most conservative criteria, exceptional cases of no influence statistically occurred in which the original diagnosis was reversed by subsequent follow-up or by additional sections. Notably, these were examples of chorioadenoma destruens in which villi were not at first demonstrated and a heavy overgrowth of trophoblastic cells simulated choriocarcinoma.

Results

Nature of sample groups and intrinsic factors. In most Asian countries hydatidiform moles are handled with other pregnancies by midwives or in the homes; this is not the case in the United States. Asian patients with choriocarcinoma, however, are more apt to come into a local hospital. Therefore an accurate ratio of choriocarcinoma to hydatidiform mole cannot be established from the figures in TABLE 1, and there is no way of estimating whether the large numbers of choriocarcinoma are due in part to a proportionately large number of hydatidiform moles.

TABLE 1
GEOGRAPHIC DISTRIBUTION OF PATIENTS IN STUDY

Geographic location	Total available	Cases reviewed	Diagnoses of cases used			
			Total	HM*	CAC*	CAD*
Total.....	1453	692	469	192	176	101
United States.....	647	195	135	82	36	17
Asia.....	806	497	334	110	140	84
Taiwan.....	148	148	92	32	33	27
Philippines.....	80	80	46	30	12	4
South Korea.....	57	57	40	24	12	4
Vietnam.....	54	54	41	8	20	13
Indonesia.....	47	47	44	—	21	23
Thailand.....	40	40	33	1	27	5
South India.....	25	25	13	2	8	3
Nagasaki.....	46	46	25	13	7	5
Ceylon.....	268†	—	—	—	—	—
Singapore.....	41†	—	—	—	—	—

* HM, hydatidiform mole; CAC, choriocarcinoma; and CAD, chorioadenoma destruens.

† Not included in report because clinical data were not available.

Patients in Asian university hospitals are drawn from, and accordingly represent, a population usually restricted to the neighboring environs only. Because of lack of transportation or communication, cases of choriocarcinoma in such hospitals are not characteristic of those screened from an entire country, as only occasional cases may be referred to the one or two pathology laboratories from outlying regions. Therefore in Asia, unlike such series in the United States, the number of cases of choriocarcinoma usually reflects a restricted population; the general occurrence is probably much more frequent than the referred sample would indicate.

Other factors that must be considered in the bias of the sample are related to the following World War II reconstruction that progressed in different countries at different rates. In some laboratories only the barest routine were possible before 1950 and the data necessarily were scattered. Generally, the numbers of cases contributed to the study increased each year.

because of improvement in clinical and pathological facilities. This increase continued, although the material observed after 1953 was not solicited.

Errors in diagnosis. A common speculation among Western practitioners unfamiliar with the work of their Asian colleagues is that the apparent frequency of trophoblastic tumors in Asia is due to differences in diagnostic criteria. However, in this study, aside from those arbitrary criteria that were necessary for statistical study, it was found that actual errors in diagnosis by Asian contributors were only slightly different in nature or quantity from those errors on cases contributed by United States workers to the Albert Mathieu Chorionepithelioma Registry, Chicago, Ill. The most common causes of errors in cases diagnosed as choriocarcinoma were syncytial changes, inadequate curettage specimens, and overgrowth of trophoblast tissue at placental site. In patients diagnosed with hydatidiform mole, cases were excluded because of insufficient gross evidence. Examples of chorioadenoma destruens or invasive mole had the highest degree of compatibility in both Asian and Western samples. Only very rarely in either group were nontrophoblastic lesions, such as atypical squamous cell carcinoma, called choriocarcinoma.

It was possible to obtain figures from Taiwan over two 5-year periods: 1948 to 1952; and 1953 to 1957. TABLE 2 shows that the numbers of cases

TABLE 2
BASIS FOR SELECTION OF CASES CONTRIBUTED FROM TAIWAN

Type and disposition	Total	Years of diagnosis	
		1948-1952	1953-1957
Reports submitted.....	148	41	107
Not retained in study.....	56	21	35
Incompatible with study diagnosis.....		14 (34%)	16 (15%)
Retained in study.....		20	72

increased as diagnostic criteria were applied in accordance with those agreed upon for this study. Of 107 cases submitted in the second 5-year period, there was only 15 per cent diagnostic incompatibility, most of this being due to lack of gross descriptions in the series of hydatidiform moles. There was some incompatibility in the interpretation of choriocarcinoma: not only those cases that should be called questionable, but also those listed as definite examples of this disease. Improvement in diagnostic compatibility resulted in the retention of 72 cases for study in the second 5-year period as compared to 20 cases in the first 5-year period.

Differences in diagnostic criteria are therefore not a factor in the apparently high incidence of choriocarcinoma in Asia.

Ethnic and geographic factors. Although initial reports indicated a high frequency of trophoblastic tumors in areas with Malayan racial mixtures

(for example, the Philippines, Thailand, and Indonesia), the final tabulation (TABLE 1) reveals that large numbers of cases also occur in Korea, Japan, Singapore, Taiwan, Vietnam and, possibly, southern India. The racial strains represent not only Malayan admixtures, as in the Philippines, Indonesia, and some portions of southeast Asia, but also include areas of relatively pure Mongoloid strains and Caucasians. The areas concerned all represent a spread of climatic conditions from tropical to seasonal, with temperatures ranging from freezing in seasonal climates to higher than 100°F. in tropical climates.

TABLE 3
AGE DISTRIBUTION OF PATIENTS BY GEOGRAPHIC AREA AND DIAGNOSIS

Age (years)	Hydatidiform mole		Choriocarcinoma		Chorioadenoma destruens	
	Asia	U. S.	Asia	U. S.	Asia	U. S.
			Number			
Under 20.....	5	12	5	6	2	5
20-24.....	23	32	26	10	18	4
25-29.....	22	17	28	7	21	3
30-34.....	25	12	30	7	12	3
35-39.....	16	4	20	4	12	2
40-44.....	7	1	9	1	10	—
45-49.....	5	—	14	1	7	—
50+.....	2	—	6	—	—	—
Unknown.....	5	4	2	—	2	—
Total.....	110	82	140	36	84	17
Average age.....	32	25	33	28	32	25
Age range.....	17-51	16-40	18-53	18-48	19-48	18-39
			Percentage			
Under 20.....	5	15	4	17	2	29
20-24.....	22	41	19	28	22	23
25-29.....	21	22	20	19	26	18
30-34.....	24	15	22	19	15	18
35-39.....	15	5	14	11	15	12
40-44.....	6	1	7	3	12	—
45-49.....	5	—	10	3	8	—
50+.....	2	—	4	—	—	—
Total.....	100	99	100	100	100	100
Average age.....	32	25	33	28	32	25
Average range.....	17-51	16-40	18-53	18-48	19-48	18-39

It is interesting to note that the sample also includes areas predominantly Moslem (Java), predominantly Catholic (the Philippines) and predominantly Buddhist (Thailand). No particular cultural habit peculiar to religious practices can be singled out as influential in the causation of trophoblastic tumors.

Within the broad terms of reference, it is concluded that no obvious relationship exists between the figures of trophoblastic tumors and specific geographic, racial, or cultural factors.

Age of patient. The ages reported for the Asian patients and for the United States patients were analyzed statistically in order to determine if there were significant differences between the two groups. The age distribution of the patients in various Asian countries was shown to be similar. These data were therefore combined and compared to age data for United States patients (TABLES 3 and 4). It is seen that the Asian women were older in each diagnostic group.

TABLE 4
AVERAGE AGES OF PATIENTS BY DIAGNOSIS AND GEOGRAPHIC LOCATION

Diagnosis and geographic area	Average age	Standard deviation*	Range of ages
HM			
Asia.....	32	± 8.12	17-51
U. S.....	25	± 5.75	16-40
CAC			
Asia.....	33	± 9.07	18-53
U. S.....	28	± 7.95	18-48
CAD			
Asia.....	32	± 8.32	19-48
U. S.....	25	± 6.87	18-39

* Approximately 68 per cent of the ages can be expected to be within one standard deviation above and below the average.

The data were tested for statistical significance. Chi-square tests, using the common median in each diagnostic group, were made with the following results: hydatidiform mole, Chi square, 21.120; choriocarcinoma, Chi square, 15.02; and chorioadenoma destruens, Chi square, 2.892.

Patients with hydatidiform mole in Asia and in the United States therefore differed at the 0.001 level of probability; patients with choriocarcinoma and chorioadenoma destruens differed between the 0.05 and 0.10 levels.

Abnormal pregnancies. An attempt was made to analyze factors related to a tendency toward abnormal pregnancies in the production of trophoblastic tumor. Abortions and stillbirths were grouped together as abnormal. In the United States, histories of ectopic pregnancies were rare. Only in the Asian women with choriocarcinoma were preceding molar pregnancies frequently recorded.

TABLE 5
OBSTETRIC HISTORIES IN ASIAN AND UNITED STATES WOMEN WITH TROPHOBLASTIC TUMORS

	Hydatidiform mole		Chorioadenoma destruens		Choriocarcinoma	
	Asia	U. S.	Asia	U. S.	Asia	U. S.
Total number with histories....	80	37	43	15	80	30
Nulliparas.....	20%	38%	14%	47%	5%	27%
Average number of pregnancies....	4.7	2.04	5.1	1.7	4.8	2.4
"Abnormal pregnancies".....	10%	15%	9%	43%	16%	28%
Abortions.....	24	5	13	6	39	13
Stillbirths.....	7	2	3	—	4	1
Moles.....	0	0	1	—	15	1
Median age (years).....	31	27	29	32	32	32

Percentage

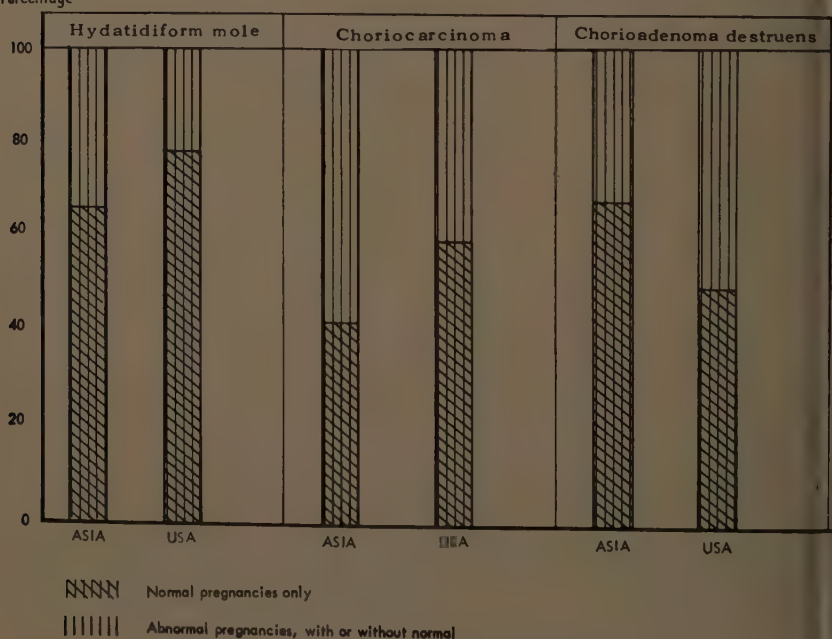


FIGURE 1. Obstetrical history in relation to diagnosis.

The past pregnancy histories of Asian women, including all pregnancies occurring one year or more prior to the diagnosis of trophoblastic tumors, were compared to those of the United States women (TABLE 5 and FIGURE 1). If these are plotted by cumulative frequencies and age at the time of diagnosis, curves more or less similar are obtained for both groups (FIGURES 3, and 4).

Although the percentages of patients with abnormal pregnancies appeared higher for hydatidiform mole and for choriocarcinoma in the Asian than in the United States group, Chi-square tests indicated that the difference could occur by chance in sampling. Conversely, the apparently higher incidence of abnormal pregnancies in United States patients with choriocarcinoma destruens was not shown to be a real difference.

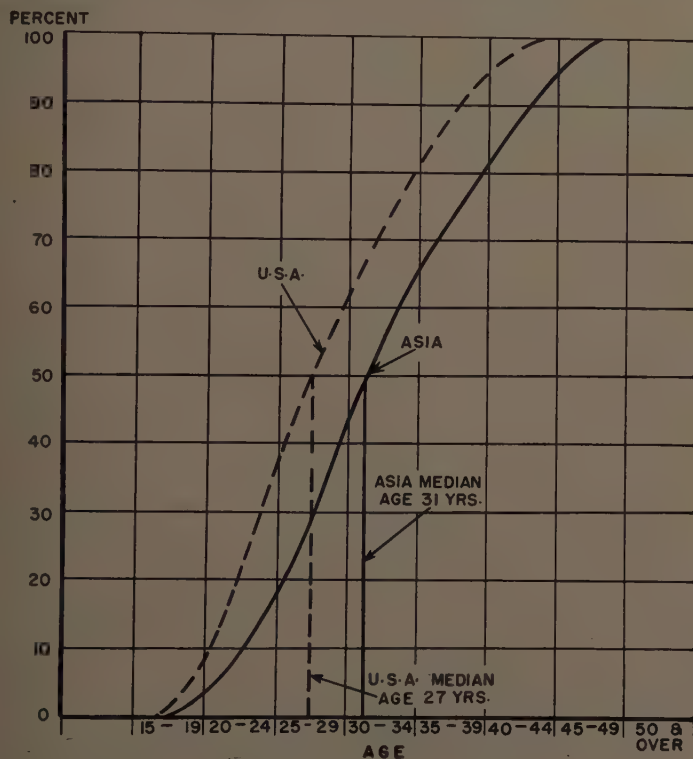


FIGURE 2. Percentages of patients with hydatidiform moles who had previous abnormal pregnancies (cumulative by age at time of diagnosis).

In neither the Asian nor the United States samples did the median ages of the patients with histories of abnormal pregnancies differ significantly from those with normal pregnancies only.

This would seem to indicate that abnormal pregnancies that are predominantly abortions in both series are not associated with the relatively higher frequency of trophoblastic tumors in the Asian women as compared to the United States women studied in this series. The absence of any significant difference in age between the two groups tends to support this conclusion.

Histopathological features. A comparison of the histopathological preparations of trophoblastic tumors in Asian women with examples from The Albert F. Mathieu Registry and from the files of the Armed Forces Institute

of Pathology showed little difference in appearance of the Western and Asian tumors. In both series, choriocarcinoma demonstrated the same variation of histological pattern and metastatic distribution. Interesting anomalies such as molar villi metastatic to the lung were noted in two Asian women with hydatidiform mole. No attempt was made in this study to grade the trophoblastic cells in any of the tumors or to quantitate the degree of histological malignancy.

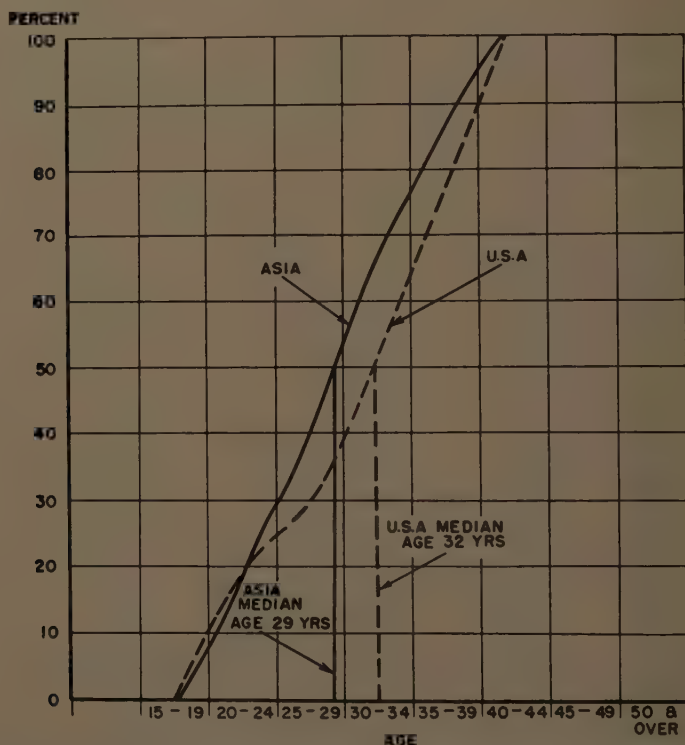


FIGURE 3. Percentages of patients with choriocarcinoma destruens who had previous abnormal pregnancies (cumulative by age at time of diagnosis).

The choriocarcinomas in 15 Asian women with a preceding history of mole were also compared to those choriocarcinomas without antecedent mole. Similarly, these 2 groups also showed no qualitative difference. The order of pregnancies was plotted in Asian women with moles preceding choriocarcinoma, but no significant correlations were found (FIGURE 5).

It was concluded that choriocarcinoma, while it is more frequent in patients with moles and apparently more frequent in Asian women, is biologically the same tumor whenever it arises in the adult female uterus, and that differences in frequency are not related to differences in the histopathologic features of the tumor.

Clinical features. Even with histories from widely separated sources, recorded clinical signs and symptoms remained remarkably similar (TABLE 6). However, there was noted a greater tendency toward anemia in Asian women with choriocarcinoma and more cases of toxemia in Asians with hydatidiform moles. These were not analyzed for statistical differences, as they were thought to represent unrelated associated findings. Similarly, due to lack of uniform data, extensive analyses of treatment and roentgen and laboratory findings were not correlated with the patient's subsequent course.

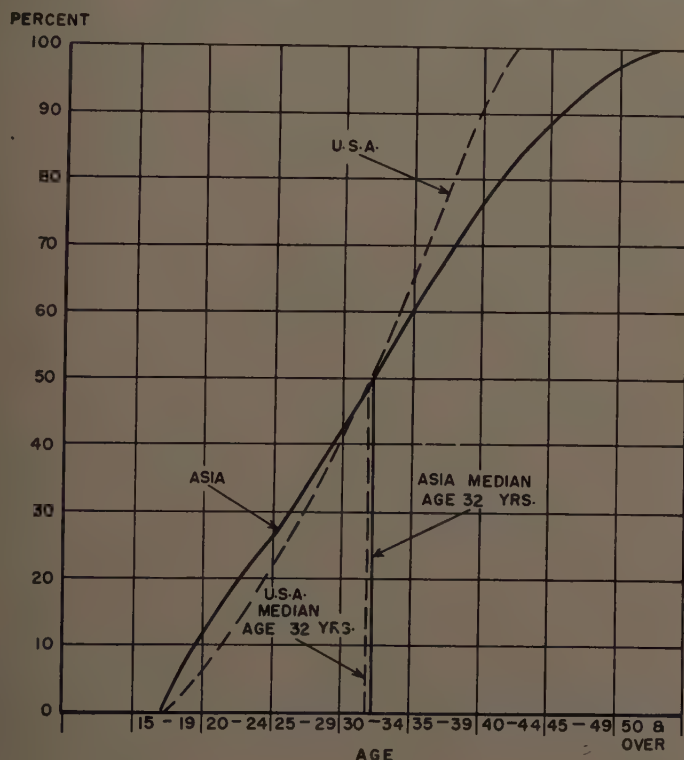


FIGURE 4. Percentage of patients with choriocarcinoma who had previous abnormal pregnancies (cumulative by age at time of diagnosis).

Duration and survival could be compared from reliable follow-up data on 57 Asian and 17 United States patients with choriocarcinoma. Survival rates were calculated by the actuarial method. The resulting curve indicates that a slightly lower percentage of United States patients died within the first year as compared with Asian patients, but that a more rapid decline in the survival rate occurred for the Asian patients thereafter (TABLE 7). These findings support the view that the Asian tumor is not relatively a more benign tumor. Differences in survival figures may be due to sampling, as the Asian women generally receive initial treatment later in the course of their disease.

Others. It is not within the scope of this paper to discuss the many interesting variations of the tumor that a large series provides. Tumors that seem to regress spontaneously were not peculiar to any geographical area. However, from our observations of autopsied cases, there is a strong indication for caution in the clinical interpretation of metastasis. Large areas of hemorrhage and edema may suddenly appear in the lung around a minute if not invisible, cluster of trophoblast cells. This "tumor" would seem to

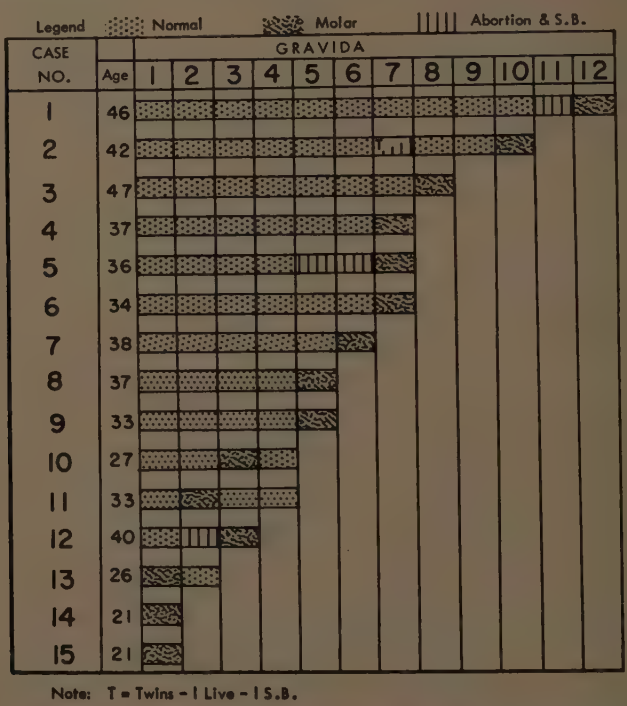


FIGURE 5. Order of molar pregnancies in fifteen Asian patients with choriocarcinoma

disappear by X ray as the hemorrhage resorbs. True regression of tumor needs to be verified histologically in every case.

The problem of nomenclature, based on the behavior of trophoblastic tumors, is also beyond the purpose of this paper, and has already been extensively studied by one of us.^{11, 12}

It was apparent from this series that, in future studies, not only the histological malignancy of hydatidiform moles but also of chorioadenoma destruens and of choriocarcinoma should be correlated with the patient's course. Clearly, the presence of a villus cannot be taken as an absolute sign of a benign tumor without a total appraisal of the manner in which it associates itself with the proliferating trophoblast, the degree of disorganization of this trophoblast, and the extent of its autonomous penetration in the uterus.

TABLE 6
CLINICAL SIGNS AND SYMPTOMS IN PATIENTS WITH CHORIOCARCINOMA

Frequency distribution	Asia		U. S.	
	Number	Percentage	Number	Percentage
erine bleeding.....	109	93	35	97
ostmolar bleeding.....	23	20	7	19
estabortion bleeding.....	15	13	6	17
st partum bleeding.....	5	4	10	28
eeding associated with abdominal pain..	16	14	5	14
tumor mass in lower abdomen or pelvis..	15	13	8	27
dominal enlargement exceeding the estimated age of gestation.....	9	8	9	30
Total patients with data.....	117		36	

TABLE 7
SURVIVAL RATES FOR CHORIOCARCINOMA PATIENTS BASED ON 57 ASIAN AND
17 U. S. PATIENTS

Duration from time of diagnosis Interval (months)	Asia Percentage Survival rates	U. S. Percentage Survival rates
0-6.....	100.0	100.0
6-12.....	47.4	58.8
12-18.....	35.2	39.2
18-24.....	26.2	39.2*
24 and over.....	18.7	39.2*

* No deaths occurred in the interval.

Discussion

This study has shown that the reported higher frequency of choriocarcinoma and of hydatidiform mole in Asia is not due to the accident of differences in histological interpretation of these tumors. Even after application of rigid histological criteria, the large series of trophoblastic tumors collected in Asia indicates the relative frequency with which these tumors appear in the routine diagnostic material of pathology laboratories in the countries represented by this study.

Hospital figures from reliable clinics in Asia also indicate frequencies of hydatid moles as high as 1 in 200 to 250 deliveries. Adjusting for incompleteness of diagnosis, the figure remains high in comparison to reported frequencies in Western hospitals.^{2, 4, 7, 8, 13, 14}

Similarly, reports of the frequency of choriocarcinoma range from 1 in 1000 to 1 in 3708. The latter figure was obtained by one of Asia's

most esteemed pathologists, who arbitrarily eliminated many probable cases, as was done in the present study.⁷ The true incidence of chorionic carcinoma in Asia probably lies somewhere between these 2 figures.

While estimates based on hospital statistics cannot be taken as true incidence, their worth as an indication of relative frequency is substantiated by the fact that the figures from many different sources cluster within comparable ranges, indicating that these reports are probably based on unselected samples of the population. The worth of these estimates is strengthened further by autopsy figures that reveal the number of deaths from choriocarcinoma proportionate to deaths from all other causes in large general hospitals. All cases were histologically verified and the autopsy frequencies are remarkably similar: 1/150, Philippines General Hospital (included in this study); 1/128, Tokyo Jikeikai Medical College;⁶ and 1/11, Department of Pathology,⁷ Hongkong University.

True incidence or rates must be based on an accurate population survey. The control and study samples must include women of the same age group and of similar socioeconomic status. The data on factors being analyzed must be obtained by similar methods. It would be necessary for such a survey to be done simultaneously in the United States on properly selected groups, preferably representing both rural and urban, and poor and rich populations. We have found that no comparable population statistics exist for countries of Asia or for countries of the West. Published vital statistics are from poorly defined populations, and statistical studies of trophoblastic tumors in the Western literature seem to be based on changing populations over a period of years.

However, since the custom of referring cases is not established in Asia and since transportation difficulties generally inhibit wide extension of laboratory services, the hospital frequencies may be supposed to represent a truer picture of the local population than is usually the case with rare diseases in the United States.

A consideration of the sample from which the present study is taken leads to the broad exclusion of race, religion, culture, and geography as factors associated with the greater frequency of trophoblastic tumors in Asian women. However, race needs to be compared further by taking a similar sample from a described area in Africa, as it has been noted by one of the contributors to the Section of Geographic Pathology of the Armed Forces Institute of Pathology; moreover, personal communications from workers in Turkey and Iraq indicate the frequent appearance of the tumor. The diagnostic material of university hospitals in these areas.

We believe that socioeconomic status is a significant factor and that future population surveys will be designed to differentiate between economic groups. That this is not the sole factor is implied by a survey³ of Puerto Rican women at Bellevue Hospital, New York, N. Y.

We have statistically demonstrated in this study that Asian women with trophoblastic tumors are significantly older than United States women in our samples. There is some similarity of age distribution in the series reported

from The Albert F. Mathieu Registry, uncorrected for race and geographical area,¹⁰ and the series of United States women in the present study, corrected for race. The statistical charts of the cases included in the present study show a large proportion of tumors appearing in Asian women in the third and fourth decades of life. In future studies, these must be correlated with the numbers of pregnancies in healthy women of corresponding age groups.

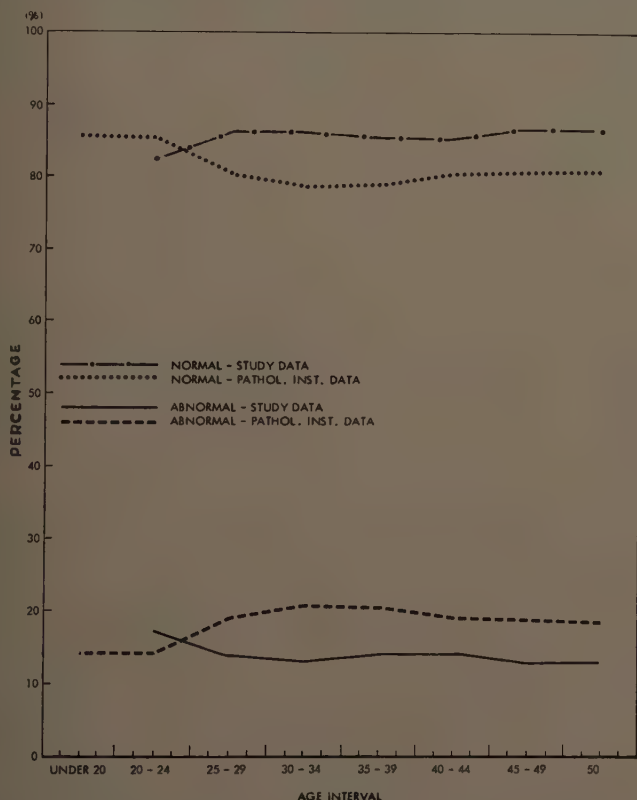


FIGURE 6. Percentage distribution of pregnancies based on cumulative total by age patient.

The frequency of abortions was considered as a possible factor in comparing our samples of Asian and United States women with trophoblastic tumors. In our data, this was found to be insignificant, even considering sampling differences due to earlier or more thorough medical attention in United States women. This was further verified by a study of 518 women treated by the gynecologic and obstetric clinics of the Taiwan University Hospital, Taipei, Formosa. This group, treated for a variety of causes, was used as a control group, and pregnancy histories were obtained. The percentage of abortions was plotted by age groups and no significant difference in this respect was found between those with trophoblastic tumors and those without such tumors (FIGURE 6).

Due to the bias of the sample, the ratio of hydatidiform moles to chorionic carcinomas was not calculated, and no comparisons were made between these ratios for Asia and for the United States. However, other data in the study indicate that the majority of choriocarcinomas in Asian women arise in patients with no history of antecedent moles. Therefore, the high frequency of moles in Asia is not the only factor responsible for a high frequency of all trophoblastic tumors.

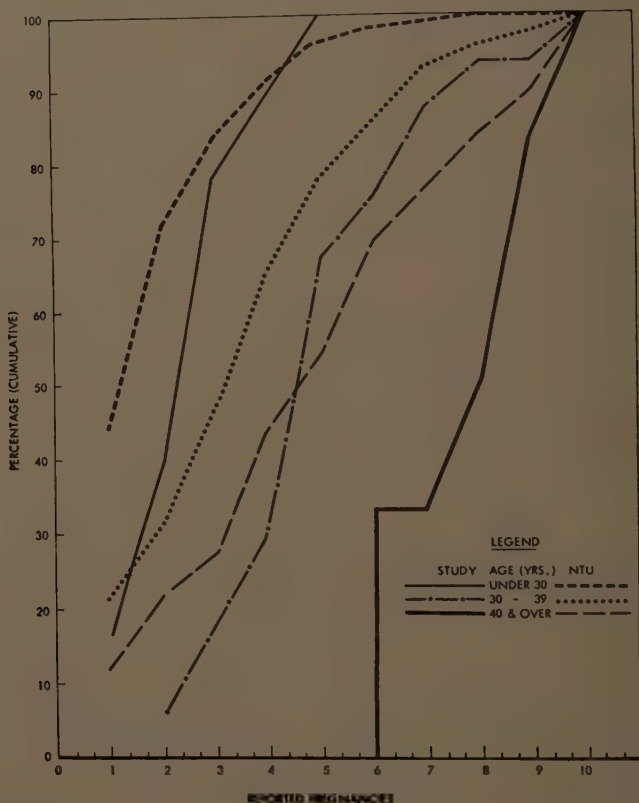


FIGURE 7. Patients distributed (cumulatively) by number of reported pregnancies. Taiwan study data compared with a random sample of patients without trophoblastic tumors (NTU).

Since the average numbers of pregnancies differ in Asian and in United States women, incidence figures must consider the ratio of tumors to total pregnancies as well as the incidence in a given population. Reports from various sources indicate that the frequency is higher in all Asian women regardless of the number of pregnancies, but that the Asian frequency also rises in older age groups. Data were obtained concerning pregnancies in women treated at the same hospital in Taiwan from which the study cases were contributed. The control group was questioned several years later.

than were the patients in the study group and therefore cannot be taken as a comparable population.

The age at which there was the maximum likelihood of having the average number of pregnancies in one of these two groups was only slightly different from that recorded for the other. However, by grouping the cases into 3 categories according to age, a larger sample was obtained. In the oldest group, a divergence occurred between the study group (Taiwan women with trophoblastic tumors) and the control group (Taiwan women without trophoblastic tumors). In the older age group there was a sharp increase in the frequency of pregnancies (FIGURE 7), a finding that will require confirmation by a study involving a larger series of cases observed simultaneously with control cases. However, as a preliminary finding, it is attractive since it can be associated logically with the over-all age differences in the Asian and the United States women, with the lower economic status of the Asian women, and with the possible alteration from healthy to an unhealthy environment for growing trophoblast by the shortened intervals between repeated pregnancies in older women.

If a higher frequency of pregnancies is found to be a causative factor, as well as a statistically associated finding, there is a strong possibility that the tumor can be eliminated by public health education.

Summary and Conclusions

Clinical and pathological material from a large sample of trophoblastic tumors was collected in Asia, and the histological criteria of diagnosis were compared with those used in large United States pathological laboratories. A high degree of diagnostic compatibility was found.

The cases were grouped according to diagnostic categories: hydatidiform mole, chorioadenoma destruens, and choriocarcinoma. A similar sample from the files of the Armed Forces Institute of Pathology was obtained by the application of the same diagnostic criteria. The two samples were compared.

Clinical and histological observations showed that the tumors in Asian and United States women behaved similarly. Degrees of histological malignancy were not compared.

True incidence figures must be obtained from a well-planned survey of the relevant populations. However, hospital statistics supported by pathological verification indicate a higher incidence of trophoblastic tumors in Asian women than in United States women. Autopsied cases of choriocarcinoma in 3 large general hospitals averaged 1 in 131 autopsies.

In these samples, the average age of the Asian patients was found to be older by statistical tests than the age of United States women in each diagnostic group.

The average number of pregnancies among Asians was consistently higher and there were many more pregnancies in the older age groups.

There was no difference in the percentage of abnormal pregnancies in Asian women with trophoblastic tumors and in United States women with these tumors. A study of obstetric histories of women in Taiwan showed

no differences in abortion histories in Taiwanese women with and without trophoblastic tumors.

A pilot study on women in the hospital clinics in one Asian country showed that women in the older age groups with trophoblastic tumors had a greater frequency of pregnancies than those without trophoblastic tumors in the same area. This finding must be verified by studying a larger group of cases.

Geographic and ethnic factors are not thought to be related to the cause of trophoblastic tumors. Socioeconomic status may be the one cultural factor requiring correlation in future studies. Frequency of pregnancy in older age groups is the most important associated finding in preliminary studies. However, the total number of pregnancies may not account for all of the cases of trophoblastic tumors appearing in Asian women.

Acknowledgments

We express our appreciation to Ethel Hicks, Medical Statistics Section, Armed Forces Institute of Pathology; Lucia Dunham, National Cancer Institute, Public Health Service, Bethesda, Md.; Hugh Grady, Seton Hall College of Medicine and Dentistry, Jersey City, N. J.; Arthur Hertig, Boston Lying-In Hospital, Boston, Mass.; T. Balasingham, Singapore; and George Cooray, Colombo, Ceylon.

References

1. ACOSTA-SISON, H. 1957. Studies on microscopically diagnosed chorioepithelioma from twenty-seven cases admitted to the Philippines General Hospital from 1950-1954. *Asiatic Congr. Obstet. Gynecol. Tokyo.* : 135-152.
2. CHEN, T. Y., T. L. PENG & SHU YEH. 1957. Chorioadenoma and Choriocarcinoma. *Repts. Inst. Pathol. Natl. Taiwan Univ. Spec. Issue.* : 54-82.
3. DOUGLAS, G. W. 1958. Personal communication.
4. HASEGAWA, T. 1957. Statistical investigations of hydatid mole and chorionepithelioma in Japan. *Asiatic Congr. Obstet. Gynecol. Tokyo.* : 130-134.
5. HIGUCHI, K. 1957. Histopathological studies in chorioepithelioma and destructive hydatidiform mole. *Asiatic Congr. Obstet. Gynecol. Tokyo.* : 153-158.
6. HIGUCHI, K. 1954. Personal communication.
7. HOU, P. C. & S. C. PANG. 1956. Chorionepithelioma. An analytical study of 22 necropsied cases with special reference to the possibility of spontaneous regression. *J. Pathol. Bacteriol.* **72**: 95-104.
8. HUWER, G. Frequency and importance of hydatidiform mole and chorionepithelioma in China. Unpublished.
9. IVERSON, L. 1958. Choriocarcinoma and related conditions in southeast Asia. *Schweiz. Z. Allgem. Pathol. u. Bakteriologie.* **21**: 581-586.
10. NOVAK, E. & C. S. SEAH. 1954. Choriocarcinoma of the uterus. *Am. J. Obstet. Gynecol.* **67**: 933-961.
11. SARWONO PRAWIROHARDJO, K. S. MARTIONO & T. SUTOMO. 1957. Hydatidiform mole and choriocarcinoma in Indonesia. *Asiatic Congr. Obstet. Gynecol. Tokyo.* : 112-129.
12. SUTOMO, T. 1955. Choriocarcinoma in Indonesia. *Schweiz. Z. allgem. Pathol. u. Bakteriologie.* **18**: 791-801.
13. UCHIDA, H. 1957. Statistical and clinical observation of chorionepithelioma malignum during ten years. *Asiatic Congr. Obstet. Gynecol. Tokyo.* : 185-203.
14. WANG, C. H., P. C. OUYANG & C. S. HUANG. 1953. A clinical study of chorioepithelioma. *J. Formosa Med. Assoc.* **52**: 565-580.

15. WEI, P. Y., P. C. OUYANG & T. L. PENG. 1957. Trophoblastic diseases at National Taiwan Univ. Hospital. A clinical study. Repts. Inst. Pathol. Natl. Taiwan Univ. Spec. Issue. : 27-52.

Discussion of the Paper

GORDON W. DOUGLAS (*New York University College of Medicine, New York, N. Y.*): Despite the great difficulty of carrying out adequate statistical studies, the work of Lalla Iverson and her co-workers leaves little doubt that trophoblastic tumors do occur with extraordinary frequency in Asian countries. The three hundred and thirty-four Asian cases selected with care from a much larger body of material, analyzed, and subjected to pathological verification by the highest authority settle beyond question the possibility that this difference is due to variation in pathological study.

Moreover, the geographic locations from which these cases were collected represent extreme differences in race, culture, and climate, which are as great as the more obvious differences between populations of the Asian countries and the United States. Realizing that the socioeconomic status of patients in the United States sample is probably much higher than in the Asian group, the authors have expressed the belief that socioeconomic factors may be significant in relation to the occurrence of trophoblastic tumors.

In this respect, figures from Bellevue Hospital, New York, N. Y., are of interest. This large municipal hospital has served, through the years, the successive waves of immigrants reaching this country, and the hospital population has been drawn from the lowest economic level in the city. By the same criteria described by Iverson, there have been 65 hydatidiform moles, 6 cases of chorioadenoma destruens, and 5 cases of choriocarcinoma since 1925. While figures for the earlier years are not available, it is estimated that this period would include 70,000 deliveries and 15,000 abortions seen in the obstetric and gynecologic services. It is clear that among the Irish, Italian, Negro, and now Puerto Rican groups of the city, the remarkable frequency of chorionic tumors observed in the Far East has not occurred even in the lowest socioeconomic groups. Moreover, an attempt to collect a large body of material from neighboring institutions in the metropolitan area resulted in the accumulation of only 250 cases from 14 hospitals, and represented patients from all socioeconomic levels over a period of 2 to 3 decades.

The difference in average age between Asian and United States women with hydatidiform mole is not confirmed by a comparison of figures with a study made several years ago.¹ The age distribution of 201 patients with hydatidiform mole was almost exactly that of the Asian series in this paper, and the agreement is particularly noteworthy in the case of women over 40, who comprised 13 per cent of patients in the Asian group and 13.5 per cent in our study.

One of the most significant points in this study is the demonstration that, in regard to pathology and clinical outcome, chorionic tumors appear to be the same entity in Asian countries and in the United States. These tumors have no counterpart in experimental animals, and their comparative rarity

has sharply limited investigation in this country. For chemotherapy testing as well as for the clinical investigation of other concepts expressed at this conference, the Far Eastern countries offer a rich source of material.

Reference

1. DOUGLAS, G. W. 1957. The diagnosis and management of hydatidiform mole. *Surg. Clin. North Am.* : 379-392.

R. HERTZ (*National Cancer Institute, Public Health Service, Bethesda, Md.*) : L. Iverson has presented entirely plausible evidence regarding possible histopathological mechanisms involved in the potential regression of metastatic trophoblastic lesions. I do not know if there is actually included in the large body of cases she has reviewed any completely documented case of fully spontaneous regression of peripheral metastatic disease in a patient with histologically proven choriocarcinoma.

H. W. EDMONDS: May I point out that Iverson's evidence of a greater frequency of hydatidiform mole in a broad geographic area is consistent with the possibility that one significant factor in the production of a mole may be a gene of nonselective value. An outstanding example of such a nonselective gene would be the familiar gene for blood group B. This gene for B is of worldwide distribution, yet it is present in a relatively large proportion of the population only in certain areas of the world—particularly is it frequent in east-central Asia, as shown in maps of world frequency of blood group genes.¹ Note that I am not proposing that there is any connection between any blood group gene and hydatidiform moles, but rather pointing to the possibility that a gene of similar nonselective character and analogous world-distribution might be a significant factor in the production of moles; if so, this could be an explanation of the interesting fact of geographic pathology that Iverson has demonstrated.

Reference

1. NEEL, J. V. & W. J. SAULL. 1954. *Human Heredity*. Univ. Chicago Press. Chicago, Ill.

W. B. OBER (*Knickerbocker Hospital, New York, N. Y.*): This may confuse the issue somewhat, but now that Edmonds raises the question of blood groups and their genes, there is one empirical observation that can be made. Human blood group A will react with biological fluids rich in chorionic gonadotropin. Serum from a pregnant woman during the peak of chorionic gonadotropin production will agglutinate A cells. No one has yet found an explanation for this.

THE NATURAL HISTORY OF CHORIOCARCINOMA: PROBLEMS OF IMMUNITY AND SPONTANEOUS REGRESSION*

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τὰ δ' ἔνθεν οὐτ' εἶδον οὐτ' ἐννέπω†

τέχνηαι δὲ καλχαντος οὐκ ἀκραντοί.

Aeschylus: *Agamemnon* 248-249.

Introduction

During the span of a normal pregnancy, the chorionic epithelium mimics the behavior of a malignant neoplasm, proliferating and invading the uterine wall and, on occasion, discharging portions of its substance into the maternal circulation. This peculiar tissue, frequently characterized as a kind of physiological carcinoma, continues to burrow into a seemingly passive and perhaps even receptive endometrium until the latter part of the first trimester, when the biological balance of power begins to shift.

Trophoblast, like its host, is a temporary thing. The life course of the fetal plasmodium has been mapped out to achieve a specific functional objective and thereafter to be cast off and forgotten. Over a period of about nine months and under the influence of factors as yet unknown, the trophoblast passes through a phase of destructive expansion into a continuously regressive state ending with delivery and the ejection of the placenta.

Certain aberrations of pregnancy also appear distinguished by trophoblastic involution. Hydatidiform mole, for centuries an inspiration to medical mystics, usually is regarded as a product of degeneration. Choriocarcinoma, a traditionally malignant disturbance, has been known to misbehave; scattered instances of spontaneous remission and cure despite grave prognosis have been reported and, in some cases, excision of the primary tumor has been followed by disappearance of metastatic disease. It is possible that trophoblastic regression, operating as either a primary or secondary factor, may participate in various other gestational disorders, notably spontaneous abortions; nevertheless, such regression, paradoxically enough, may be an indispensable element in the termination of a physiological pregnancy.

During the past half century the bizarre life cycle of trophoblast has been

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† What passed next, I saw not, nor can show,
Save that the prophet's lore must be fulfilled.

(*The Agamemnon of Aeschylus*, Tr. J. C. Lawson, Cambridge University Press, 1932, 17.)

celebrated in an awesome mass of literature, yet our understanding of choriocarcinoma has been graced by few dramatic improvements upon the speculations of F. Marchand and his contemporaries. Concrete experimental attacks have been exceptional and generally inconclusive. What survives is a tangled confusion of vague clinical histories, equivocal diagnoses, semantic altercations, and fragmentary researches. One wishing to explore the field must be prepared, in essence, to follow a frayed and interrupted thread through a bibliographical labyrinth that has neither beginning nor end. The basic problems of trophoblastic regression remain a playground for the imagination. The answers simply are not known.

Thus we shall not resolve the questions of trophoblastic growth and involution, but rather attempt to describe some of the more provocative theoretical and experimental aspects of these and related phenomena. The possible roles played by mechanisms of immunity in the regulation or rejection of fetal tissues will be discussed, and broad avenues of future inquiry will be suggested in the hope that eventually a more rigorous literature may be founded on new and more accurate information. Understanding of trophoblastic disease requires study of the healthy counterpart, and we shall touch briefly upon relevant attributes of the normal and pathological physiology of the placenta. In the long view, the natural history of choriocarcinoma is inseparable from that of the trophoblast itself.

The Problem of Spontaneous Regression

From the time that choriocarcinoma was first recognized as a discrete clinical entity, it was observed that not all tumors of this type seemed to arise in the uterus. In some patients, lesions were found in the vagina or even more distant sites, yet the uterine lumen was free of disease. A flurry of case reports entered the literature about the turn of the century, and similar histories have been published sporadically in succeeding years. The papers by Dunger¹ and Hörmann² in 1905 probably stand among the first to describe the spontaneous regression of vaginal metastases after the removal of primary choriocarcinoma in the uterus. More recently the status of current knowledge of such involution has been reviewed with respect to both choriocarcinoma³ and hydatidiform mole,⁴ so that a practical perspective on available clinical information may be achieved.

At the outset any effort to understand trophoblastic regression must surmount purely conceptual barriers. What is a choriocarcinoma, and why should it be malignant? Although ideas of classification have become somewhat clearer in late years, it is still difficult to know exactly what was represented by older accounts of "chorionepithelioma malignum," or "benignum," for that matter, and related pathology. Documentation has been sparse and occasionally debatable. One may single out the case described by Grein⁵ as a chorionepithelioma malignum, with a claim of spontaneous recovery in the face of malignant disease; from his own drawings, showing the penetration of the uterine tissues by villi, one may deduce that the fortunate patient was a victim of invasive mole, not choriocarcinoma. The case reported by Clemmer and Hansmann,⁶ to be discussed below, is similar.

In sum, one may wonder whether the commonest cause of spontaneous regression in choriocarcinoma has not been pure misdiagnosis.

Another bothersome question is that of regression itself. What is regression? In appraising the clinical course of a patient, how does one establish that a wholly fortuitous degeneration has occurred? Such a query may appear somewhat naïve until one looks closely at the published reports of *Spontanheilung*. Chest films have been a comparatively recent and insufficiently applied aid to diagnosis, but a radiograph nonetheless does not carry the authority of a tissue section. The occasional reference to regression in patients treated with irradiation or chemical agents does not make sense, and it hardly establishes spontaneity. The term has been applied loosely to many cases in which necropsy findings revealed degeneration in focal or metastatic tumor nodules. Here it is reasonable to speak of local regression, but not of spontaneous healing. The case of Prym⁷ shines as an example of semantic confusion. In this report of fatal metastatic choriocarcinoma in the male, with demonstrable fibrosis of the primary testicular growth, the author refers to *Spontanheilung*. When reading accounts of so-called spontaneous healing, one ought to bear in mind the admonition of little Buttercup that things are not always as they seem; it is necessary to distinguish between local and systemic events, especially in the situation of a healed tumor and a dead patient.

These problems notwithstanding, there have been well-documented cases in which disease vanished for no apparent reason and the patient recovered. If true spontaneous regression occurred, one may wonder why a potentially lethal growth should have shifted course so strikingly. Unfortunately there are no ready answers to account for regression, and one speculation very probably is as good as another. In recent years, however, certain areas of inquiry have come to appear imminently more rewarding than others, and this discussion will be limited to one of them: immunity.

The relation between regression and immune mechanisms may be examined from several aspects. Clinical toxemia often has been thought to show at least superficial resemblances to states of allergy and, indeed, has been treated with antihistamines; some cases of threatened abortion have been managed similarly. Experiments to create antibodies against syncytium and reproduce the syndrome of eclampsia are as old as the side-chain theory of Paul Ehrlich that inspired such efforts. In the near past, there has been some evidence that the fetal connective tissues may contain antigenic materials, and one may wonder whether trophoblastic regression may not depend upon some kind of intrauterine autosensitization analogous to erythroblastosis fetalis.

The broad area of tissue transplantation between hosts of differing genotypes promises to yield a vast quantity of useful knowledge, but presently must be dealt with on essentially hypothetical levels. Trophoblast and its tumors are not genetically identical with the host tissues, except in the male. A pregnant woman carries within her body the cells of an alien animal, her sexual mate, who can approach no more intimately than homology. Does spontaneous involution of trophoblast, whether at the end of a normal

gestation or in the case of tumor degeneration, represent a host reaction of quite the same sort encountered in the sloughing of a skin graft? The recent discovery that tissue transplants may be rejected in far greater frequency when made from male to female than when attempted in the reverse direction is extremely provocative, and will be considered in its application to the study of chorionic neoplasms.

From an epistemological standpoint, it does not matter whether one blames the regression of a choriocarcinoma on a presumptive host allergy, so long as immunologically oriented reveries do not diverge appreciably from observed fact. Woodger⁸ has pointed out that biological metaphors may serve useful functions provided that they are recognized as being only figures of speech and, indeed, his amusing parable of the four islands illustrates how easily a common objective may be approached from several directions at once. Thus the examination of trophoblastic regression may start with a possibly unwarranted assumption: that immunity against trophoblast, its products, or its neighboring tissues can and does come to exist and play a definite role in regressive events. Once granted, this premise may serve as a convenient substrate for mechanistic interpretation and perhaps furnish a supplantable scaffolding upon which a more lasting structure may be erected.

It is appropriate to begin by inquiring how the mother might become sensitized against the fetal tissues and, in particular, how antigenic agents might manage to reach the sites of antibody production.

MECHANICS OF SENSITIZATION

Paraplacental Routes

During recent years there has been increasing evidence in favor of the transfer of immunologically active substances from mother to embryo by pathways other than through the placental barrier. Brambell, in his monograph of 1951,⁹ as well as in later publications, has implicated amniotic fluid as a significant carrier in animals. Although present attention is limited to placental function in sensitization, the suggestive findings of Brambell, together with the possible corollary of reverse transfer, deserve at least passing mention.

Diffusion at the Placental Site

Anatomico-physiological considerations. Perhaps the most obvious route by which a mother might become sensitized against trophoblast or other fetal tissues is through direct contact between maternal blood and syncytium in the intervillous space. Blood of the mother, washing over the chorionic epithelium during the extent of even a prematurely terminated pregnancy, conceivably might carry off substances either actively expressed by the trophoblast or leached out by the moving stream.

The actual degree of contact would appear to be more than minimally adequate to provide generous amounts of antigen to the mother. The total villous surface of the placenta undoubtedly is enormous and describable

only in terms of powers of ten. Computations of the gross area at term have ranged from about 7 sq. m.¹⁰ to about 15 sq. m.;¹¹ the estimate of Christoffersen,¹² based on a survey of 61 placentas and yielding an average of roughly 14.5 sq. m., may represent par for the technique. When one takes into account the vast increase in surface area contributed by the microvilli of the syncytial brush border, well demonstrated in recent electron microscopic studies,^{13, 14} the probable true figure transcends imagination. The rate of blood flow over the villi has been estimated as somewhat less than 5×10^{-4} cu. mm./sq. cm. of villous surface/min.¹⁵ and, undoubtedly, is slow enough to allow liberal exposure. This value, however, may be interpreted differently, since extraction might be presumed more efficient in a rapidly moving stream.

In addition to contact area, diffusion must be reckoned in terms of path length, in this instance, the interval between maternal and fetal circulations, at the very least the combined thickness of the syncytial and endothelial layers. In the latter part of pregnancy, despite impressions gained from conventional microscopy, greatly flattened Langhans' cells often intervene between the syncytial plates and fetal capillary endothelium.¹⁴ The minimal intervacular separation has been given as 3 to 6 μ ;¹³ hence the intrinsic permeability of cellular membranes, together with whatever selectivity may be superimposed by active cytoplasmic function, is likely to prove a more significant element in transfer activity than physical path length alone.

Whether potentially antigenic substances may be borne across the placental barrier by processes of active transfer is not known. The passage of antibody from mother to fetus is well established, and one may conjecture that both carbohydrate and protein agents are capable of traveling in opposite directions. The role of intracytoplasmic syncytial droplets in antigen carriage is difficult to evaluate, and one may argue eternally the question whether such inclusions represent pinocytosis or merely local secretion.

The free diffusion of antigenic materials across the placental site probably is limited to the immediate area of the intervillous space; the fetal and maternal tissues usually are set off from one another by a local deposition of fibrinoid substance, the well-known layer of Nitabuch, which may behave somewhat as an immunological no man's land, walling the fetus off from chemical interaction with its host.

Antigenic discharge following trophoblastic injury. Any trauma that disrupts the integrity of the chorionic epithelium will prevent selective trophoblastic transfer at the site of injury and will also leave the underlying villous stroma exposed, allowing leakage into the maternal circulation. The mechanism of autoextraction used by Schneider¹⁶ to account for gestational problems of coagulation may be extended to the leaching out of possible antigens in the core of the villus. Severe lacerations involving fetal capillaries may permit the drainage of their contents into the intervillous space, an event to which certain diseases of hypersensitivity, notably erythroblastosis fetalis, may be attributed. Wiedow,¹⁷ in 1888, had called attention to the apparent correlation between placental lesions and nephritis in pregnancy, suggesting that hypothetical nephrotoxic materials might find access to the maternal

circulation following fetal death; this concept will be accorded further consideration in a later section. In numerous placentas from the latter half of seemingly normal pregnancies, the villi have been claimed to show syncytial defects exposing the underlying stroma, which was covered by recent or old clots containing fetal blood cells.¹⁸ The same investigator has reported the occurrence of such thrombi, with nucleated blood cells, in two thirds of specimens seen in erythroblastosis. Syncytial and other age changes appear to be accelerated in toxemia,^{19, 20} and may be ascribed to focal hypoxia²¹ or, in the loosest sense, to some type of hyperimmune reaction. The appearance of toxic agents in fluids perfused through placentas subjected to artificial ischemic insult²² may reflect localized damage to vascular endothelium, permitting the escape of stromal contents; possibly such leakage might follow ischemic injury to syncytium as well.

Trophoblastic Deportation

The removal of chorionic tissue from the placental site and its dissemination in the maternal vascular tree offer a ready mechanism for sensitization. A deported trophoblast, if at all antigenic, might exert such function once carried to sites favorable for the stimulation of antibody synthesis. The detachment of syncytial tags or even entire villi through denudation of the chorionic connective tissue will permit simultaneous egress of soluble materials from the ground substance of the villus, thereby affording two routes of sensitization for the price of one.

In his now famous monograph on eclampsia,²⁵ published in 1893, Schmorl described the occurrence of multinucleated cellular fragments in the smaller pulmonary vessels of eclamptic women who had died during their seizure or shortly after delivery. Because these intravascular masses seemed morphologically identical with *in situ* syncytium, as well as with decidual giant cells, he named them *Plazentarzellen*. His drawings and photographs, shown in FIGURE 1, survive comparison with contemporary material. Schmorl claimed to have found embolic placental cells in the intervillous space, in myometrial vessels, and in fresh heart blood, thus tracing out the essential route of maternal deportation. Inasmuch as embolic syncytium was seen in the lungs of 14 out of 17 cases of fatal eclampsia, and not at all in 4 non-eclamptic *post partum* deaths, Schmorl supposed this syndrome germane to the disease, and somehow related to its pathogenesis.

In 1902, Schmorl²⁶ presented 3 additional cases of placental embolism in *post partum* death. These patients had not suffered convulsions, but necropsy findings showed widespread parenchymal hemorrhagic necrosis. In another series of 158 gestational and puerperal deaths^{27, 28} he reported numerous placental emboli in the lungs of 83 cases diagnosed as eclamptic, but only exceptionally in women dying of other causes. In the same publications, Schmorl also described 3 cases involving syncytial transport to the lungs in molar pregnancy.

The observations of other investigators were variable. Lubarsch²⁹ claimed to have found syncytial emboli in the lungs from only 5 cases of a total of 14 deaths in eclampsia. Syncytial embolism was reported by Pels Leusden³⁰

in 2 instances of toxemia complicated by lobular pneumonia; in 1, he claimed to have found a free villus within a deep myometrial vein, perhaps anticipating the later work of Veit. The series of Kassjanow,³¹ however, revealed no significant difference between normal and eclamptic pregnancy, and the author postulated that placental embolism was an ordinary and physiological occurrence during gestation; in this assertion he very likely commands priority.

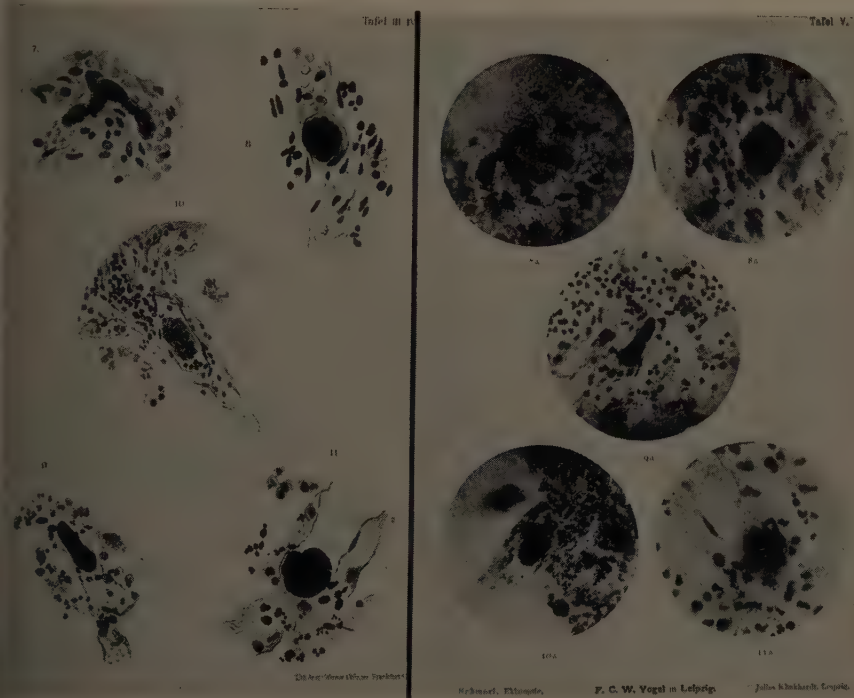


FIGURE 1. Illustrations from the classic monograph on eclampsia, published by Schmori in 1893, with examples of pulmonary embolism by *Placentarzellen*. Original drawings by author at left, with photographs of same microscopic fields at right.

From time to time the reader may find references to so-called liver cell emboli, especially in the earlier literature. Evaluation of such reports is extremely difficult, although it is conceivable that many of the observed tissue fragments may have been trophoblastic in origin. In one of the cases described in this paper, a mole with pulmonary metastasis, some of the ectopic trophoblast strongly suggests hepatic parenchyma (FIGURE 7). Similarly, the confusion once enveloping bone marrow cell emboli,³² often mistaken for syncytium, has been resolved with their recognition as megakaryocytes; the cell forms sketched by Aschoff, illustrated in FIGURE 2, are consistent with megakaryocytic embolism as understood by most latter-day pathologists.

The term deportation was coined by Veit³³ in 1901, in connection with an ectopic pregnancy with penetration of tubal vessels by villi, most of which had maintained connection with the placenta proper. In some areas (FIGURE 1) he found villi apparently set free and floating within the maternal veins. The observations of Veit were soon confirmed in similar cases, as reported by Hitschmann^{34, 35} and Fellner.³⁶ In the original communication and in



FIGURE 2. An historical view of trophoblastic deportation. (a) The pulmonary giant cell emboli reported by Aschoff in 1893 and considered by him to have originated in the bone marrow. Consistent with the cell forms and "naked nuclei" now recognized as megakaryocytes. (b) Uterine sinus from placental site in eighth month of pregnancy, as sketched by Friedländer in 1870, showing red blood cells and multinucleated giant cells enmeshed in a fibrin thrombus. (c) Another figure from Friedländer, with several cells isolated from such a sinus. (d) *Zottenverschleppung* in the case of ectopic pregnancy reported by Veit in 1901. Intravenous villi claimed to lack connection with the placenta proper. a, courtesy of Springer-Verlag, Berlin, Germany; d, courtesy of Ferdinand Enki Verlag, Stuttgart, Germany.

subsequent papers,³⁷⁻⁴³ Veit took the position that deportation was a normal process occurring during any and all pregnancies, with particularly marked frequency in gestational disorders.

Several years earlier Neumann⁴⁴ had reported myometrial deportation in a case of mole. Veit³⁸ assumed that similar transport might be observed in placenta accreta, placental polyp, and in premature separation. Williamson,⁴⁵ writing in 1903, considered syncytial embolization in choriocarcinoma analogous to the deportation of normal tissue, echoing after a fashion the prior opinion of Poter and Vassmer⁴⁶ that deportation per se did not necessarily connote malignancy.

The terminological chaos beclouding the classification of trophoblastic tumors had its counterpart in the concept of deportation, a word that appears to have signified all things to all men. One suspects that Veit originally intended to imply only a displacement of placental villi from their normal situation in the intervillous space by simple penetration of the maternal venous apertures. Veit often failed to distinguish between free and attached villi, and the problem of interpretation was immensely complicated by a gradually mounting enthusiasm for the term *Zottendeportation*, which came to be used for the embolization of almost any placental component and its transport to the maternal lung. When the paper of Dunger¹ appeared in 1905, it contained a footnote by Schmorl roundly condemning the loose employment of this word and asserting that only cellular emboli, and never entire villi, had been found in the maternal lung. For the term then in vogue, the critical reader of the older literature might supply *Zottendeportation ohne Zotten* and perhaps find himself nearer the truth.

Syncytial embolism in normal pregnancy has been described from time to time, and now is accepted generally as a common event with no somber overtones. Most pathologists dealing with obstetrical material are familiar with the appearance of pulmonary syncytial emboli, yet, as pointed out by Park and Lees,³ no survey comparable in scope to that of Schmorl had been released by 1950, so that his observations and interpretations remained neither substantiated nor refuted. Aside from the recent report by Park,⁴⁷ the problem of when and why trophoblast is deported has not advanced much beyond the level of understanding that prevailed in 1905.

In an attempt to gain a clearer picture of the nature of deportation, a statistical breakdown has been performed upon a broad sample of maternal fatalities at the Boston Lying-in Hospital, Boston, Mass., during the years 1930 through 1957. Autopsy material, representing the lungs, uteri, and cervixes from a total of 109 cases, was screened for the presence of trophoblastic emboli. Excluding perhaps one dozen cases with incomplete histological files, this sample embodies all maternal deaths in the specified interval. The specimens were collected without discrimination against any cause of mortality. Each of the authors screened all sections personally, and findings were cross-checked. The entire series was evaluated in terms of the types of emboli observed, together with the salient pathological features noted in the tissues. As a further bar to prejudice, each reviewer worked without reference to the original autopsy records, noting his personal impressions of disease seen in the slides; these diagnoses were then compared with findings in the hospital files.

Detailed results of the survey are presented elsewhere.⁴⁸ It may be remarked that of the 109 cases, 57 showed definite trophoblastic embolism, including the 2 examples of villous transport mentioned previously; 11 cases were suggestive and probably positive; 8 were questionable; and 33 cases appeared completely free of deported trophoblast. Bone marrow embolism was noted in 4 cases, and material from the amniotic fluid, such as squamæ, in 2. In 5 patients, the pulmonary vessels contained occasional nucleated blood cells identified as normoblasts and possibly of

fetal origin. Syncytial particles have been found in the lungs of undelivered pregnant women who died as early as the fourth month and, in at least 3 cases, the extent of embolism was sufficient to have been a primary cause of death, a syndrome reported by Marcuse,⁴⁹ Trotter and Tieche,⁵⁰ and Arnold and Bainborough.⁵¹

These findings support the contention of Schmorl that trophoblastic embolism appears to be a regular event in eclampsia. In terms of recorded pathological diagnoses, pulmonary embolic syncytium occurred frequently in eclampsia (13 of 16 cases), pre-eclampsia (2 of 3 cases), and toxemia (3 of 3 cases). It was also common in aborted pregnancies (5 of 6 cases), and massive hemorrhage (2 of 3 cases).

The series included 2 cases of choriocarcinoma and 1 of hydatidiform mole, as well as a single patient with Hodgkin's disease. These were the only instances of true or debatable neoplasia of a type that might mimic benign deportation.

Mechanisms of Deportation

Embolization of placental tissue. In normal pregnancy, as indicated in the autopsy series described in the preceding section, embolization of syncytial fragments occurs in about half of individuals surviving to term without toxemic complications. This frequency corresponds with the figure reported by Park,⁴⁷ who found pulmonary deportation in 53 of 120 pregnant women investigated post-mortem. It is highly probable that such emboli represent knots or tags of syncytial trophoblast torn away from the surface of the chorion and carried off in the maternal circulation. Whether the vacuolation that Kollmann⁵² described in syncytial excrescences and attributed to cytolysis actually contributes to their abscission may be debated; very likely, this histological effect was identical with what is now regarded as a manifestation of hormone synthesis⁵³ or, possibly, even pinocytosis.^{14, 54, 55}

Deportation may be an exclusively mechanical process, for trophoblastic buds occasionally show constrictions facilitating avulsion, as illustrated in FIGURE 3. The origin of emboli in detached syncytial knots was assumed by Lubarsch²⁹ and Ulesko-Stroganowa⁵⁶ shortly after the issuance of Schmorl's monograph, and now is considered axiomatic.

Penetration of the maternal veins by entire villi was postulated by Virchow⁵⁷ as early as 1856, and the subsequent separation of villi from the placental body in ectopic tubal implantation was demonstrated in 1901 in the famous case of Veit.³³ The phenomenon was studied closely by his contemporaries and reported in cases of normal and tubal pregnancy, placenta accreta, and hydatidiform mole.^{30, 34, 35, 44, 46, 58-60} In our own specimens, villi have been seen crowding through venous apertures in the decidual plate. FIGURE 4 illustrates invasion of a peripheral maternal vessel, perhaps the often-debated marginal sinus, by a cluster of placental villi; this specimen was obtained from a normal term pregnancy. This pattern, first described by Fa  e Scheffer⁶¹ in 1896, probably constitutes *Zotten deportation* or *Zottenverschleppung* as conceived by Veit himself, and is shown to good advantage in preparations by Boyd⁶² and Ramsey.⁶³ It is not difficult to

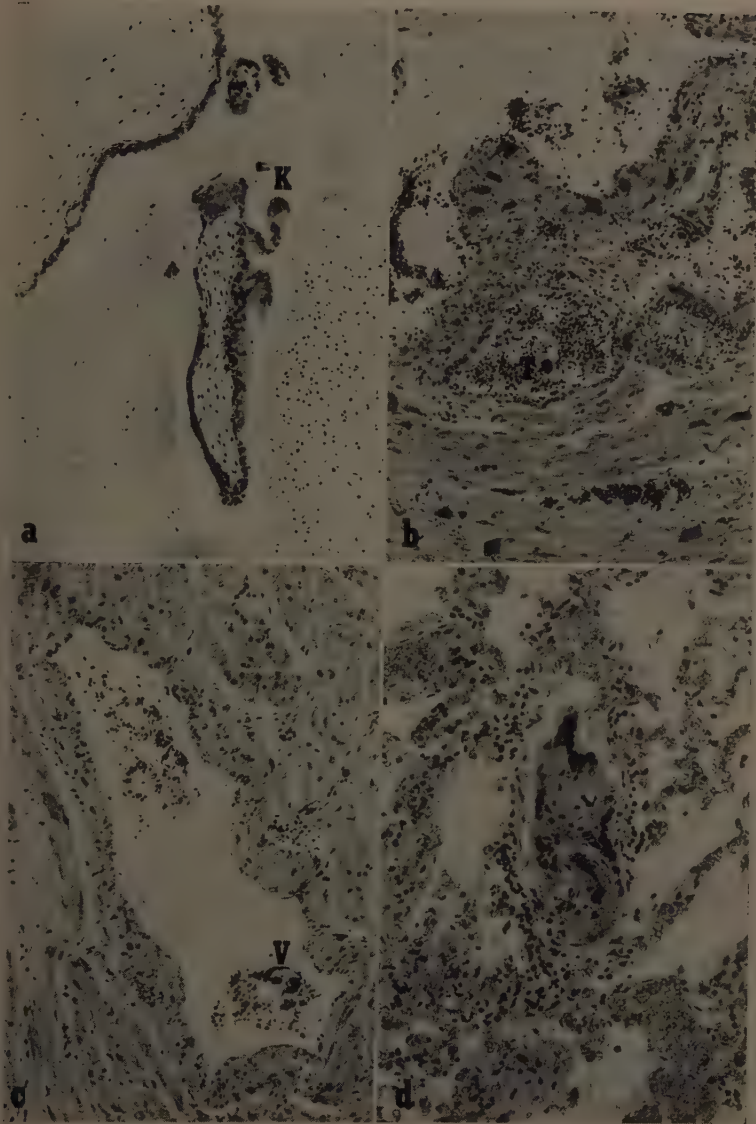


FIGURE 3. The topology of trophoblastic deportation. Successive stages in the transport of placental tissue in the maternal circulation. (a) Molar villus *in situ*, from a 19-year-old Para I, Gravida II with a history of pre-eclampsia. *K*: proliferative syncytial knot with constricted neck, likely to become detached from the chorionic tissue. This kind of configuration in a placenta would predispose to syncytial deportation. (b) Embolic syncytium (*T*) within superficial blood vessel of decidua basalis. From undelivered patient who died in the seventh month of gestation with eclampsia, pyelonephritis, and bronchopneumonia. (c) Subserosal myometrial venous sinus from same patient as in (b), with deported terminal villus at *V*. (d) Syncytial sheet in the lung of a patient who died *post partum* with pulmonary congestion, cerebral edema, glomerular degeneration, and multiple parenchymal hemorrhages. From Bardawil *et al.*⁴⁸

imagine how an external force sufficient in magnitude to alter uterine contour, perhaps even obstetrical manipulations during labor, might place the decidual and chorionic plates of the placenta in shear, so that those villi which have become impacted in the basal veins would be torn loose and permitted to float away in the uterine vessels.

In normal pregnancy, the deportation of whole villi probably occurs only with extreme rarity. The many references to villous embolization scattered throughout the early German literature appear founded on fancy rather

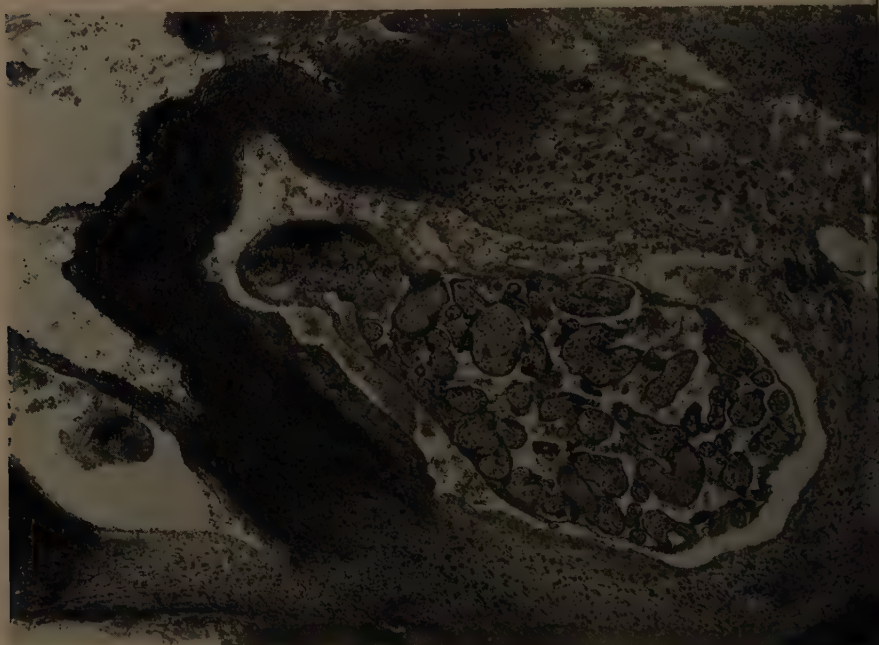


FIGURE 4. Classic *Zottendeportation*. Cluster of placental villi penetrating the maternal sinus of a normal term specimen. From Bardawil *et al.*⁴⁸

than fact, and it will be recalled that Schmorl,¹ in 1905, asserted that villous transport, although often talked about, in point of fact had never been demonstrated. The crystal ball, unfortunately, has remained cloudy through the years. In various editions of his well-known text, Novak⁶⁴ has remarked " . . . the process of deportation of trophoblastic masses and villi to the lungs . . . probably occurs in all normal pregnancies." In 1950 Kistner and his colleagues⁶⁵ claimed "the transportation of placental villi . . . as a normal incidental finding in many autopsies done on the recent parturient." Engle⁶⁶ has mentioned the discovery of entire bits of villi in the maternal circulation in tubal pregnancy; no specimens were presented and it is possible that he had in mind only local deportation of the type observed by Veit one-half century earlier. One writer has referred to the

transport of villi as well authenticated,⁶⁷ and a year later as only a possibility.⁶⁸ Some reviewers have credited Poter⁶⁸ with the description of maternal embolism by chorionic villi; it must be pointed out that his series of seven cases was restricted to hysterectomy specimens and revealed deportation only within the endometrial and myometrial vessels, thus serving as a contemporary verification of the findings published by Veit in the preceding year. Although whole villi may metastasize in cases of hydatidiform mole, a vigorous search of the literature has failed to unearth a single adequately documented example of villous deportation to the lung during normal pregnancy, and so this frequently discussed but never demonstrated phenomenon has come to seem as much a mythical beast as James Thurber's unicorn in the garden.

The occurrence of two clear-cut cases of pulmonary embolism by entire placental villi in normal gestation has been a gratifying by-product of the autopsy series described in an earlier section. Systematic search of the histological files has yielded two specimens of true pulmonary *Zottendeportation*, each in a different patient, and both, curiously enough, during the same year. The microscopic fields are presented in FIGURES 5 and 6.

One patient, a 24-year-old white Para III with a history of 2 successive miscarriages in 1930, was delivered by low forceps on March 5, 1932 after a painful but otherwise unremarkable labor. She went into shock with a progressive rise in pulse and temperature and with falling blood pressure, but with little blood loss. The patient failed steadily, ultimately expiring 36 hours after delivery. Necropsy revealed a *Bacillus welchii* septicemia, with focal renal and hepatic necrosis, acute rectal ulcerations, multiple petechial hemorrhages, and right adrenal necrosis associated with thrombosis of the ipsilateral ovarian and adrenal veins. In a large vessel of the lung, a single terminal villus was captured in transit, and is illustrated in FIGURE 5.

The clinical history of the other case was not available. Autopsy records describe a 31-year-old white female with evidence of congestive heart failure, renal glomerular degeneration, multiple visceral hemorrhages, and cerebral edema. There was a reference to hyperemesis gravidarum, but no additional information about her clinical course. The pathological report mentioned the presence of syncytial cells in the lungs. Review of the sections has revealed a small and well-preserved chorionic villus lodged in one of the smaller pulmonary arterioles, as well as a fragment of deported syncytium associated with nucleated red blood cells (FIGURE 6).

The deported villi shown in these figures were the only samples of clear-cut transport to the maternal lung observed in the more than 100 patients investigated, despite an extremely meticulous search. One other villus was seen in a deep myometrial venous sinus, just within the serosa (FIGURE 3). The patient, a 34-year-old Para II, Gravida III, died on January 28, 1947 in the seventh month of an undelivered gestation. The autopsy record, which mentions eclampsia, lists pyelonephritis, pulmonary atelectasis, bronchopneumonia, and cerebral edema as salient findings. Renal disease was regarded as the essential cause of death. The lungs of this patient were

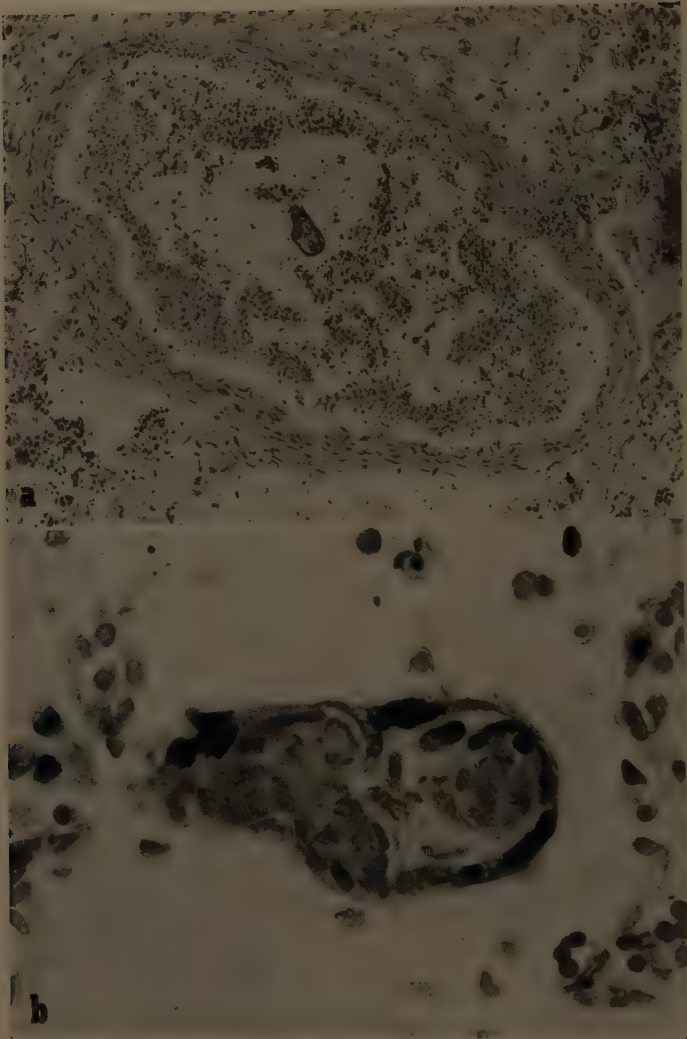


FIGURE 5. Placental villus deported to the maternal lung during normal pregnancy. Patient died of septicemia 36 hours following delivery. (a) Low-power field illustrating situation of villus within a pulmonary blood vessel. (b) Same villus at higher magnification, revealing well-preserved architecture. From Bardawil *et al.*⁴⁸

riddled with syncytial emboli of varying size, 1 of which could be traced through 3 successive serial sections. Decidual giant cells were abundant.

It is conceivable that placental site giant cells, by penetrating the walls of uterine vessels, may provide an additional source of deported syncytium. As early as 1896, Ulesko-Stroganowa⁵⁶ had published sketches illustrating such invasion in a pregnant uterus of 2½-months' gestation age. Direct vascular entry, long known to occur in the formation of the intervillous space

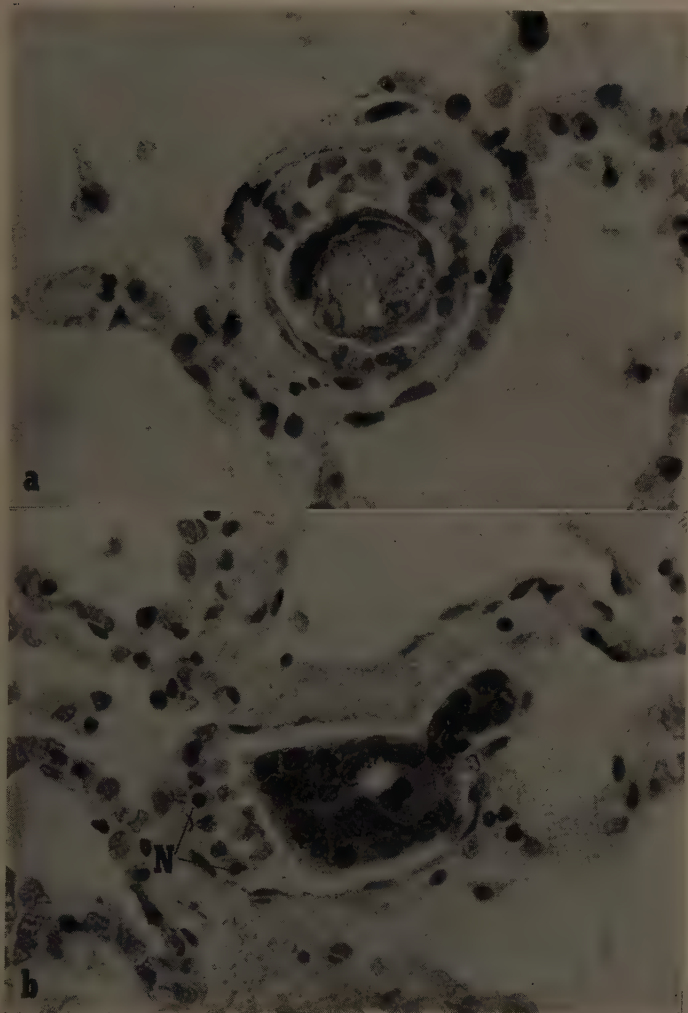


FIGURE 6. Two forms of pulmonary deportation, seen in a patient who died with glomerular disease, multiple parenchymal hemorrhages, and cerebral edema. (a) Entire terminal villus, with intact structural detail. (b) Syncytial embolus, molded to contour of vessel in which it was impacted. Note cytoplasmic vacuole. N: two nucleated red blood cells, possibly of fetal origin following traumatic exposure of placental vessels during deportation. From Bardawil *et al.*⁴⁸

as well as in metastatic or ectopic chorionic sites, suggests another means by which potential antigens might become available to the maternal reticulo-endothelial system throughout the course of pregnancy.

Whether swelling of pulmonary capillary endothelium as a result of stimulation by the presence of foreign matter in the blood stream, with a consequent narrowing of vascular bore, actually assists in the entrapment of

deported tissue, as postulated by Siegmund,⁶⁹ may be argued endlessly. Very likely the majority of embolic fragments are sufficiently large to become impacted in capillaries without the intervention of a *deus ex machina*.

Careful observation of maternal necropsy material will be rewarded by the discovery of deported emboli corresponding to most stations along the anticipated pathway from the uterine lumen to the lung, including vessel-lumen within the decidua and myometrium (FIGURE 3). Because such emboli, once within the gradually widening venous channels, are swept away to the right heart and the lung, one may expect to find tissue particles caught up almost exclusively in the pulmonary capillaries. This appears to be the case in the clinical material, and also in the animal studies described below. Transport of chorionic material to more distant sites, such as the brain, depends in all probability upon anastomotic connections between the uterine and vertebral veins, recently demonstrated by Jeffcoate,⁷⁰ or upon the occasional presence of a patent foramen ovale. The direction of flow in these vessels may be a function of intrapelvic pressure, and perhaps may be influenced by trauma or clinical manipulations. In any event such paradoxical deportation may be considered relatively infrequent.

Hemodynamic factors. The mode of circulation through the intervillous space constitutes still another variable in the etiology of deportation, yet it cannot be outlined with certainty. For more than twenty years the concept of Spanner,⁷¹ deduced from injection preparations and verified by later workers employing similar techniques,^{72, 73} has enjoyed general acceptance. Spanner assumed a centrifugal flow originating within the central cotyledons and passing up through the subchorial space to drain by the marginal sinus. Recently this concept has been challenged by observations implying that the marginal sinus may be a fiction.^{62, 63} It has been suggested that maternal blood both enters and leaves the intervillous space within the pale of each cotyledon, and that the relative distribution and degree of mixture are determined by local pressure gradients. Clearly, the invoked pattern of fluid movement is dynamic and inconstant, so that any given line of flow behaves according to a form of uncertainty principle and can be analyzed only by statistical criteria. Under the old schema, one may think of a traumatic shearing injury to peripheral villi, with consequent egress of tissue fragments by way of the marginal sinus. Thus, adherents to the theory of Spanner may anticipate frequent embolization from the most outlying cotyledons, with but little originating in the central areas hemmed in by septa, except after a vigorous physical disturbance. votaries of the newer concept, denying the existence of a circumferential sinus, might prefer to imagine a central embolization of detached trophoblast, due perhaps to either direct or contrecoup displacement of the chorionic plate.

The true picture of the fluid mechanics of deportation depends in part upon what one understands by a marginal sinus. Large vessels, often apparent on gross inspection of a specimen, can be seen in the placental border, and rarely may be found to contain insinuating villi even in normal pregnancy (FIGURE 4). Such venous channels do not correspond to the size implied in the drawings of Spanner; can they properly be called marginal

sinuses? For all practical purposes, the question is academic, and these vessels may be named as the investigator desires.

Clinical Deportation

The character of trophoblastic deportation in both normal and pathological pregnancy is protean, and the biological activity of any given embolus is difficult if not impossible to assess or predict. It is convenient to classify clinical deportation according to a system based upon broad anatomical and physiological considerations. In approximately half of the women who die during or shortly after a normal gestation, the lungs are found to contain varying numbers of syncytial emboli. In our experience, no definite isolated Langhans' cells have been seen. While entire villi may be deported to the lung during pregnancy (FIGURES 5 and 6), such transport is probably an exceedingly rare event in the absence of violent trauma. Molar embolization may involve the deportation of bits of syncytium (FIGURES 7 and 8) or whole villi (FIGURE 10). The abduction of anaplastic or partially organized tissue is largely characteristic of choriocarcinoma, although here again evidence of pure syncytial deportation frequently may be discovered.

In the establishment of a working taxonomy with clinical application, we have divided observed clinocopathological patterns of deportation into the following classes: (1) deportation of cellular elements, singly or in showers, and (2) deportation of large tissue masses, either unorganized configurations or well-differentiated structures, which are either benign or malignant.

In the first class, the tissue emboli may be syncytial, cytotrophoblastic, or both, and may vary greatly in number. With infrequent exceptions, deported tissue observed in the lung consists only of syncytial fragments detached from their parent villi and embolized. When only a few bits are deposited in the lung, in all likelihood there are no clinical manifestations. In some cases, however, the pulmonary vessels may be flooded with large numbers of emboli, leading to classic obstructive symptoms such as cor pulmonale. The over-all effect, in most instances, may be regarded as subacute to chronic, and of gradual onset. Large-scale syncytial embolism may be considered a probable cause of death by massive occlusion of the pulmonary vasculature. This picture of right-sided heart failure and massive syncytial embolism during normal or disturbed gestation has been reported as a concomitant of normal pregnancy^{49, 51} and mole,⁵⁰ and may be thought of as a definite clinical syndrome. In the Boston Lying-in series discussed previously, three cases suggesting massive trophoblastic deportation and congestive failure were noted.

Syncytial embolism in a molar pregnancy may be illustrated by the following cases, presented in abstract and fully documented elsewhere.⁴⁸ A 17-year-old white girl denying pregnancy was admitted to hospital with a uterus enlarged to the umbilicus, no perceptible fetal parts, and nodular shadows in her lung fields. During a transfusion she went into shock and died 4 hours later. At necropsy, the uterus was found filled with typical molar tissue, and syncytial emboli were noted in the smaller pulmonary vessels. The principal diagnosis was cor pulmonale. Two representative emboli are



FIGURE 7. Massive pulmonary embolism in a 17-year-old primipara with hydatidiform mole. (a) Gross specimen, showing uterus filled with typical molar tissue. (b) Vesicle, with characteristic edematous stroma and trophoblastic proliferation. (c) Syncytial embolus in pulmonary vessel. (d) Metastatic tissue in lung. Note morphologic resemblance to hepatic parenchyma, suggesting so-called liver cell emboli of the older literature. Courtesy of J. M. Turner, C. B. H. Turner, H. L. Turner, D. J. Turner.

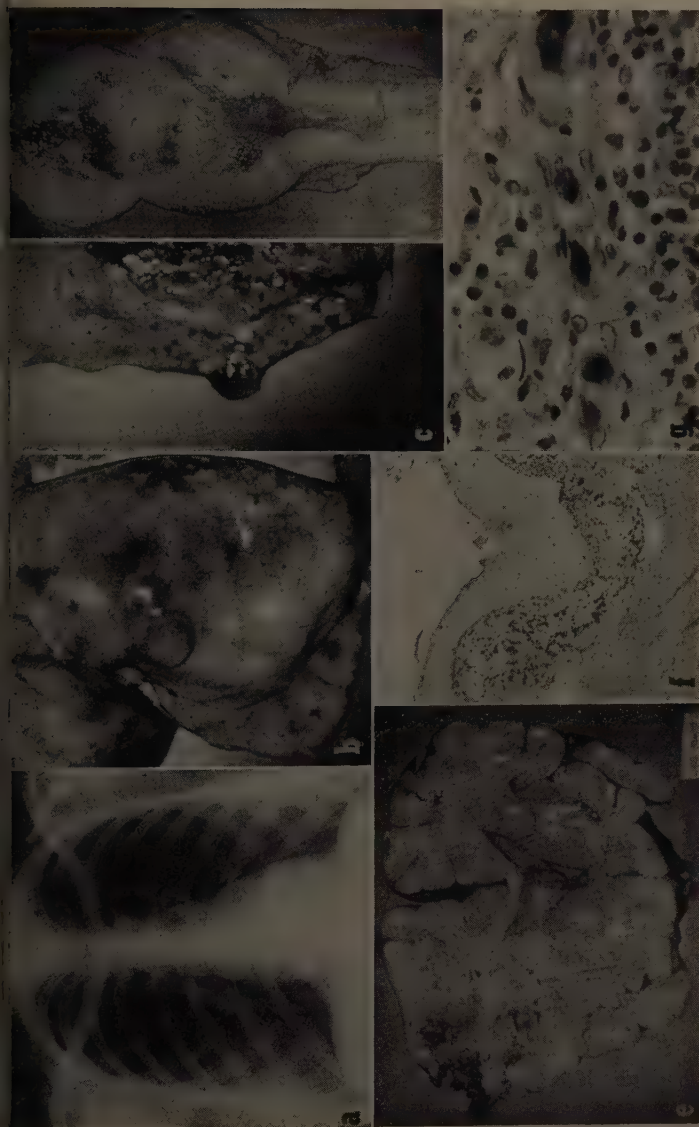


FIGURE 8. Molar deportation in a 21-year-old primigravida who died of cerebral hemorrhage probably related to trophoblastic embolism. The lungs contained numerous bits of metastatic trophoblast in varying states of organization. (a) Chest film, showing metastatic nodules. (b) Surface of lung, with bulging molar metastases. (c) Cut surface of lung in profile, showing protrusion of a molar nodule. (d) Low-power view of molar tissue in lung, indicating partial organization. (e) High power photomicrograph of pulmonary capillary containing five trophoblastic fragments. Note pleomorphism and hyperchromasia. Courtesy of E. F. Latham, Station Hospital, Quonset Point, R. I., and A. E. O'Dea, Office of the Chief Medical Examiner, Providence, R. I.⁴⁸

shown in FIGURE 7. It will be observed that one of these contains a binucleate cell suggesting the so-called liver cell fragments frequently mentioned in the older German literature.*

The following two cases typify the deportation of pure or mixed trophoblastic elements in association with other tissue components. The degree of organization or differentiation may be partial or complete; in the latter situation it is evident in the transport of entire villi.

On admission to the Station Hospital, Quonset Point, R. I., a 21-year-old white Para 0, Gravida I presented a uterus too small for the stated menstrual age, elevated gonadotropin titers, and suggestive apical shadows in chest films. She passed a typical mole estimated at 7 months, and a curettage revealed only decidual tissue. The patient was discharged 2 days later but died suddenly 2 days thereafter. At autopsy, the cause of death appeared to be cerebral hemorrhage apparently unassociated with trophoblastic cells. The lungs contained many hemorrhagic masses, some with molar vesicles, and also a number of discrete trophoblastic cells within small vessels (FIGURE 8). Metastases were associated with scarring and granuloma formation.†

A 30-year-old woman with a history of 6 prior miscarriages treated by curettage had been infertile since the passage of a mole 4 years previously. In her last illness, she presented high gonadotropin titers and symptoms implying pulmonary metastasis. Earlier thoracotomy and subsequent removal of the uterus and adnexa had revealed no trophoblastic tissue. Her sudden death seemed due to massive embolism by a tumor thrombus impacted in a pulmonary arterial bifurcation. The mass showed a necrotic center with identifiable syncytial and Langhans' cells along the periphery. The relation of the thrombus to the maternal tissues is illustrated in FIGURE 9, which indicates extensive organization and inflammatory change. The deported masses in this case conceivably may have been carcinomatous.‡

Published reports of the embolization of molar vesicles are not rare, and 2 of the more interesting accounts may be mentioned. In 1923, Miller⁷⁴ offered the history of a girl of 18, whose disease had been diagnosed as neurotic mole. Vaginal bleeding terminated in fatal hemorrhagic shock. At autopsy, tumor nodules were found in the lung, bladder, and vagina, with typical mole in the uterus. Although the vaginal wall had been invaded by masses of chorionic tissue, including villi, none was observed in vessels. The lungs contained large syncytial cells enveloped in a granulomatous reaction, as well as encysted molar villi in the nearby maternal tissue. The final diagnosis was invasive mole.

Thirty years later Clemmer and Hansmann⁶ reported the case of a 29-year-

* Boston Lying-in Hospital, CS-57-123; Good Samaritan Hospital, Lebanon, Penna., A-57-56. Courtesy L. M. Tanner.

† Free Hospital for Women, Boston, CS-58-20; Office of the Chief Medical Examiner, Providence, R. I., No. 1800; courtesy E. F. Latham, Station Hospital, Naval Air Station, Quonset Point, R. I., and A. E. O'Dea, Office of the Chief Medical Examiner, Providence, R. I.

‡ Boston Lying-in Hospital, CS-58-51; St. Francis Hospital, Santa Barbara, Calif., A-58-17. Courtesy A. J. McQueeney.

old single woman admitted with persistent vaginal bleeding. Until about 7 months before her presentation, she had led a vigorous sexual life. Her last period occurred two months thereafter, and was followed by more or less continual hemorrhage. A curettage revealed only hyperplastic endometrium. One month later the patient returned with further pain and bleeding, and necrotic trophoblastic tissue was found in the cervical canal. Her condition declined steadily until her death after another month. The uterus at autopsy contained typical choriocarcinoma. As shown in a clear



FIGURE 9. Organized tumor thrombus from patient dying with massive pulmonary embolism. Tissue is extensively organized, with barely discernible trophoblastic elements in last stages of degeneration along periphery of mass. Courtesy A. J. McQueeney, St. Francis Hospital, Santa Barbara, Calif.⁴⁸

photograph, a pulmonary mass was found to enclose a definite villus associated with thrombosis. Other degenerated villi were observed in the vicinity; the tissue was considered distinctly retrogressive.

The specimen illustrated in FIGURE 10, one of 2 metastatic masses removed from the right lung of a 21-year-old Para I, Gravida III delivered of a mole by curettage, shows remnants of transported vesicles. The villi are necrotic and surrounded by a marked inflammatory process. Two years after hysterectomy and thoracotomy the patient was alive and well. This case is a textbook example of villous deportation in hydatidiform mole, and has been reported in detail* by Coe and Reed.⁷⁵

* Boston Lying-in Hospital, CS-57-52; Minneapolis Memorial Hospital, Minneapolis, Minn. No. 22154. Courtesy J. I. Coe.⁷⁵

Although the deportation of larger emboli of mixed constitution will ordinarily be reflected in a clinical syndrome which is slow in onset and development, exceptionally the impaction of sizable masses in the pulmonary vessels may result in sudden death, a fate that seems to have befallen one of the patients reported in this section. Her demise was probably attributable to a vasomotor reaction of the type usually associated with postoperative massive pulmonary embolism. In most instances there will be a subacute or

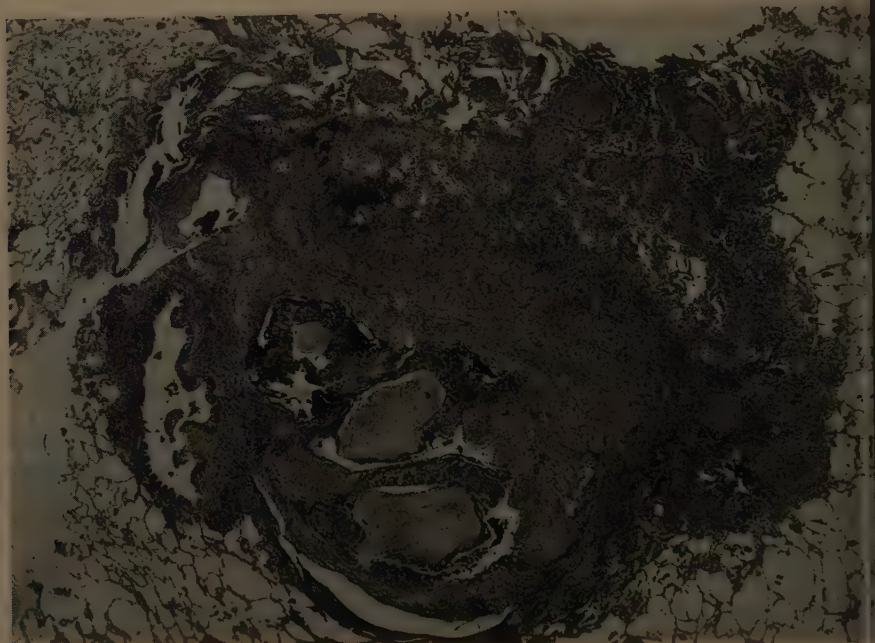


FIGURE 10. Metastatic molar tissue in lung. Remnants of villi showing degenerative changes, with local hemorrhage. Note vigorous inflammatory reaction in vicinity. This nodule, from the right middle lobe, is the larger of two identified on chest films, and appears to have been regressing at the time of surgery. Courtesy J. I. Coe, Minneapolis Memorial Hospital, Minneapolis, Minn.^{75, 48}

chronic course. Either the embolic tissue, if intrinsically benign as in the case of normal or molar trophoblast, will undergo gradual resorption over an extended period, or if inherently malignant, as in choriocarcinomatous metastases, the impactus will cause local hemorrhage and necrosis. In either event radiographic examination may be expected to reveal discrete nodular densities that may increase or decrease in size depending on their origin and growth potential. Very often the major portion of a dense area noted in a lung field may be due to associated hemorrhage and tissue reaction rather than to the bulk of the trophoblast itself. Harrison,⁷⁶ in a case of choriocarcinoma reported in detail elsewhere,⁷⁷ has shown that there will very likely be radiographic evidence of metastatic disease that has progressed

sufficiently to cause concurrent vascular disturbances such as cor pulmonale and congestive failure. In the deportation of syncytial fragments, however, one should not anticipate diagnostically useful chest films in the absence of clinically apparent pulmonary congestion and edema.

Experimental Deportation

When Schmorl first noted the presence of multinucleated cells in the pulmonary capillaries of women who had died during or shortly after eclamptic seizures, he reasoned correctly that the emboli had come from the placenta. Wondering about the possible role of ectopic chorionic tissue in producing the disease syndrome, Schmorl in effect created a state of artificial deportation by the intravenous injection of animals with saline placental suspensions.²⁵ Two rabbits given 8 cc. of homologous tissue emulsion by ear vein died in convulsions within 5 min. A pregnant rabbit, given 6 cc., delivered 2 stillbirths and 2 living offspring which died soon after; the mother expired a day later. A fasting dog, given 25 cc. intravenously, convulsed but recovered. Autopsy 5 days thereafter revealed focal infarcts in the liver, but Schmorl recognized that this of itself did not prove anything with regard to eclampsia. In the same work, Schmorl described the finding of lesions suggesting human eclampsia in a pregnant rabbit that had died in spontaneous convulsions, including pulmonary embolism by multinucleated cells and the presence of 6 fetuses, 3 of which were macerated, in the uterus.

Several years later Maximow,⁷⁸ in a survey of 40 pregnant rabbits, could find no emboli referable to the placenta. After manual kneading of the uterine horns in 5 pregnant animals, however, autopsy disclosed widespread necrosis and hemorrhage, partial or total placental separation, and pulmonary embolism by giant cells containing up to 50 to 70 nuclei, similar to those described by Schmorl. In a more recent communication, Schneider⁷⁹ has reported pulmonary thromboembolism by fibrin masses enmeshing occasional giant cells, following manipulation of the pregnant rabbit uterus.

Lubarsch⁸⁰ criticized the work of Maximow on the technical ground that the negative results of the latter were due to an inadequate histological workup. In a series of 20 rabbits injected intravenously with tissue from lung, liver, and placenta, Lubarsch claimed consistent embolism by bone marrow cells. It is impossible to interpret his experiments clearly, for one may wonder how he went about assigning given emboli to one source or another and, in particular, how he distinguished between placental and marrow cells.

Perhaps the most thoroughly conceived attempt to simulate trophoblastic deportation has been published by Park⁴⁷ in 1958. Normal, nonpregnant mice were treated with estrogen, progesterone, and chorionic gonadotropin, and then injected with saline suspensions of placenta, liver, and kidney. In no case was active growth or vascularization noted. Most emboli degenerated steadily, showing pyknosis, loss of cellular detail, and other signs of breakdown. Calcification at one site was found 150 days after operation.

Pretreatment with estrogen or progesterone appeared to prolong survival somewhat in nonpregnant animals, although gonadotropin had no visible effect. Embolic trophoblast could be found up to 65 days before complete destruction, and its fate did not seem dependent on pregnancy in the animal. Host response to placental fragments was considered sluggish or virtually absent, while renal and hepatic tissue emboli were greeted with an active inflammatory reaction. Park has referred to antecedent unpublished work in which 6 rabbits, injected with homologous placental suspensions, showed gradual autolysis of embolic matter with no apparent host response.

In an unpublished series that we carried out in 1955 in collaboration with T. C. Washburn, similar attempts were made to duplicate trophoblastic deportation. An immature 8-week human placenta was obtained, minced and homogenized, and made up to a 33 per cent suspension in saline. This mixture, in 0.5 cc. doses, was injected in the marginal ear veins of 6 rabbits. The animals were sacrificed by air embolism in lots of 2, after periods of 2, 4, and 6 days following treatment. Complete autopsies were done on all rabbits. Numerous trophoblastic emboli, in varying states of preservation, were found in the lungs of each animal. Both syncytial fragments and pieces of villi were seen, as might be expected. In no case was there evidence of active proliferation. All emboli appeared to be degenerating; indeed, in the 6-day animals the deported trophoblast was surrounded by vigorous granulomatous reaction, probably foreign-body responses. Numerous eosinophils were seen nearby; this observation, however, must be taken with caution, because of the close resemblance between eosinophils and neutrophils in the rabbit. Typical tissue reactions, including active inflammatory response, are shown in FIGURE 11.

By using experimental samples of at least 20 animals, and a nonpregnant control batch of 80, Park has given his results more than chance significance. The lack of host response in his animals, injected with homologous placenta, strongly suggests that in our own smaller series the inflammatory reaction was directed against some trait peculiar to the human tissue as such, rather than against its content of trophoblast or other placental material. Carrying the investigation a step further and trying autologous placenta would prove valuable; clearly the use of the host's own tissues would bear more directly on what happens to deported trophoblast in a pregnant woman. Pregnancy, however, represents a special state somewhere in the hazy range between tissue autology and homology, and it must be emphasized that the immunological reactions of the mother against products of conception should not be equated with those shown against other tissues in her body.

The implied increase in survival after hormone treatment, as described by Park, is extremely provocative. Park's present findings, though hardly clear-cut, definitely justify extensive investigation of the relationship between trophoblastic activity, both *in* and *ex situ*, and controlled blood hormone levels. From time to time one may encounter nebulous reports of the treatment of choriocarcinoma with this or that endocrine agent. Such accounts have been consistent only in their inconsistency, and they underscore the need for further study.

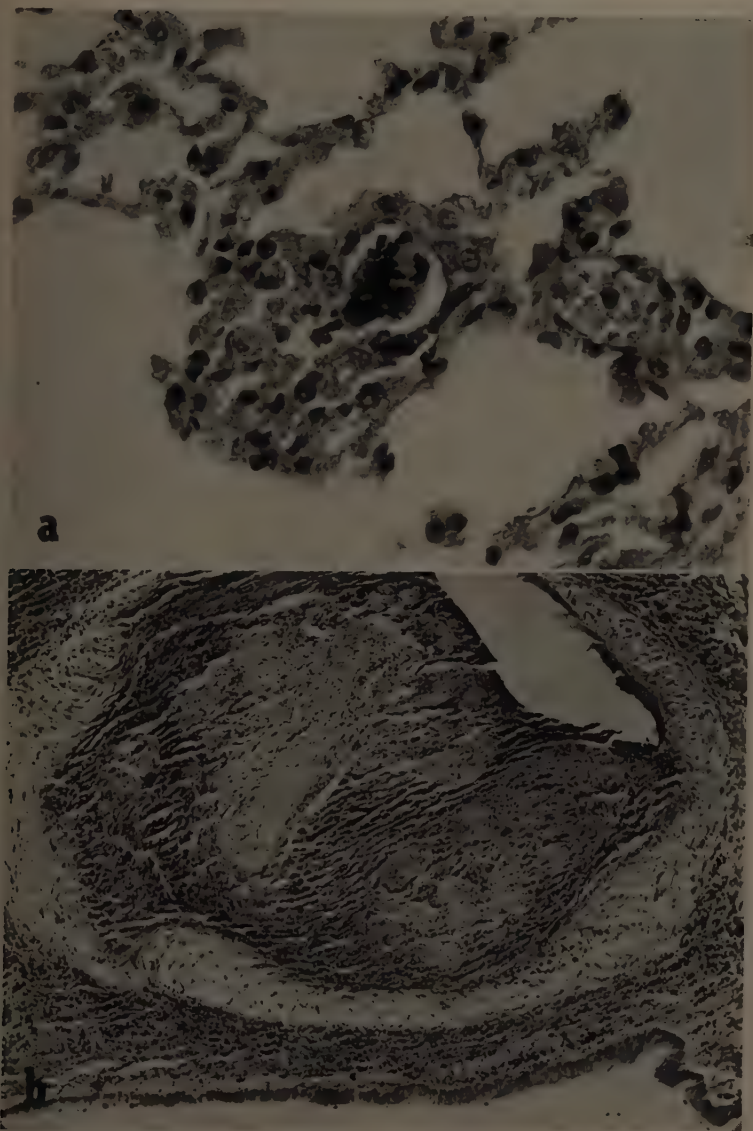


FIGURE 11. Experimental trophoblastic deportation in the rabbit, following intravenous injection of minced normal 8-week human placenta in saline suspension. Fate of tissue after pulmonary embolization. (a) Syncytial embolus immediately after injection near vein. Impaction in pulmonary capillary. (b) Degenerating fragments of embolic syncytial tissue on sixth day after injection. Note vigorous host reaction.

CONSEQUENCES OF TROPHOBLASTIC DEPORTATION

Intrinsic Growth Potential of Trophoblast

What happens to deported trophoblastic tissue will depend partially upon whatever innate growth capacity may reside in the cells themselves. Tissue culture has provided the most direct means of assessing this quality but unfortunately, evidence of the successful culture of placental explants has been meager and, indeed, not always easy to interpret. In 1929 Friedheim⁸² showed that syncytial elements did not appear to proliferate *in vitro*, but rather that any perceptible growth might be attributed to Langhans' cells. The cytotrophoblast has been given full credit for the secretion of gonadotropin in cultures of both normal^{82, 83} and molar⁸⁴ placenta. In 1933 Uyeno⁸⁵ claimed the successful cultivation of choriocarcinoma. In our laboratory cultures of placenta from various stages of pregnancy have not yielded an unequivocal reproduction of chorionic epithelium; instead, they yielded motley configurations of stellate and epithelioid cell forms, implying some degree of dedifferentiation. It may be that trophoblast can multiply vigorously, if at all, only when supplied with some form of extraneous factor or organizer available in the body fluids but not in synthetic media.

Transplantation of tissue to a heterologous host provides the severest test of autonomous growth potential. In the experiments of Kido,^{86, 87} human placenta was claimed to survive up to a week in the rabbit eye, and even to produce gonadotropin. Kido's illustrations leave no doubt that he had a viable "takes," but these are not sufficiently detailed to tell one very much about individual cell forms. In the mouse, Frowning⁸⁸ has reported the complete failure of homologous transplants, while it has been claimed that autotransplants to the anterior chamber have remained viable up to a month in both mice⁸⁹ and rabbits.⁹⁰ Fawcett and his colleagues^{91, 92} have transplanted homologous segmenting ova to the anterior chamber and removed the capsule in mice. Although there was evidence of yolk-sac formation, the embryos did not produce differentiated organ systems and, after about 10 days, they seemed to consist mainly of enormous giant cells up to 100 μ in diameter. Flourishing trophoblast appeared to suppress vascularization of the implants, and the growth of the ova seemed self-limiting.

Toolan^{93, 94} has shown that cortisone may be used to prevent host reactions against heterologous tumor transplants. Applying the technique to nonneoplastic tissues, Handler and Yerganian⁹⁵ have reported active proliferation of human placenta transplanted to the cheek pouches of hamsters maintained on cortisone. Although we have employed dose levels verging on the heroic, we have been unable to reproduce their results. In our animal series, sterile minced placenta has been inoculated in the hamster cheek pouch, both as free pieces extruded through trocars and also in saline suspensions. The implants invariably degenerated and evoked local granulomatous reaction, even when *in vitro* cultures of the same tissue survived. It will be recalled that human placenta injected in rabbits fared no better, and that the experimental homologous deportation in rabbits⁴⁷ was followed by constant

gression. Certainly the observed natural history of placental transplants has contributed little to any case that might be made for trophoblastic autonomy.

In human pregnancy one has to deal with several situations that might be regarded as simple autotransplantation, physically if not genetically. Since Friedländer²³ first described the placental-site giant cells in 1870, these curious decidual fauna have been recognized as a common finding in normal pregnancy. Their origin and function are unknown. It is possible that they may represent syncytial residua of the earliest trophoblastic invasion of the endometrium, and therefore their life cycle is of considerable interest. Estimates of giant cell survival after delivery have varied widely. Teacher⁹⁶ claimed to find giant cells in the decidua up to 25 days *post partum*, while Rutherford and Hertig⁹⁷ have observed them in a specimen obtained six weeks following delivery. The placental-site giant cells show degenerative changes in most *post partum* uteri, as shown in FIGURE 12, but whether their continued existence depends upon some definite humoral agent peculiar to the pregnant state has not been established.

Trophoblastic growth does not appear to require the shelter of the uterine cavity, since an ectopic implantation in the parietal or visceral peritoneum can remain viable throughout the course of a normal gestation. Because of the danger of uncontrollable hemorrhage, an abdominally rooted placenta is often left behind with the expectation that it will be resorbed in time. In the intraperitoneal pregnancy, a positive Friedman test was obtained up to 5 days after cesarean section;⁹⁸ although palpable remnants of ectopic placentas have been identified as long as 4 years after delivery,⁹⁹ the outer limits of actual viability are not known. In a specimen recovered 32 months after section and delivery,¹⁰⁰ architecture was still quite intelligible, but the tissue as a whole seemed necrotic.

Pathological events analogous to so-called intrauterine blighted ovum probably occur from time to time within the peritoneal cavity; indeed, the case reported by Lazarus and Schifrin,¹⁰¹ in which seemingly benign implants of chorionic villi were found in the omentum and several loops of gut, raises the possibility that in rare instances a primary choriocarcinoma might arise in the abdomen and constitute a difficult diagnostic problem. One may wonder what would have been the destiny of these ectopic villi had they not been excised.

The results of experiments in pseudoparturition have shed at least a dimming light on the question of trophoblastic growth potential. In 1935, Newton¹⁰² showed that in the pregnant mouse, following destruction of the fetus, the placenta remained viable until approximately the correct period of gestation had expired, whereupon delivery took place and was followed by estrus in one or two days. In the monkey *Macaca mulatta*, which has a 28-day menstrual cycle and 6-month gestation, it was found that placentas survived up to 3 months after surgical removal of the fetus and then were expelled spontaneously.¹⁰³ One may doubt that the placenta proper contains any physiological timing mechanism of its own, and it is tempting to speculate whether temporal regulation of pregnancy and its termination



FIGURE 12. Regression of placental-site giant cells in normal pregnancy. (a) Low power view of giant cells in peripheral portion of placental site, shortly following delivery. Cells appear well preserved. Hysterectomy performed because of uterine rupture. (b) Two decidua giant cells on fourth day *post partum*. Note degenerative changes in many nuclei, as well as cytoplasmic inclusion with halo in cell at left. This patient died with acute streptococcal endocarditis, and showed no evidence of pulmonary deportation. From Bardawil *et al.*⁴⁸

may not depend upon some central agency. The nature of the processes that control and schedule the course of gestation is still unknown, and it constitutes a challenging and fertile area for investigation.

The Fate of Deported Trophoblast

In normal pregnancy the destiny of deported trophoblast would seem to be inevitable degeneration. In most cases bits of syncytium may be found in all stages of decay, from essentially intact knots through varying degrees of fragmentation and lysis (FIGURE 13). Ordinarily no inflammatory reaction is observed, and deportation becomes clinically apparent only through the symptoms and signs of vascular obstruction in the lung. In the series reported above, morphologically intact and probably viable emboli have been seen in pulmonary capillaries as late as the tenth *post partum* day.

The picture in hydatidiform mole and choriocarcinoma differs somewhat. Excised metastases are often surrounded by inflammatory changes, implying some type of host reaction. Clinical regression in molar pregnancy is well established^{4, 104} and it correlates with the granulomata noted histologically. Representative specimens have been shown here. The cases of Miller⁷⁴ and of Clemmer and Hansmann⁶ are classic.

Similar effects in choriocarcinoma were described by Risel¹⁰⁵ and Teacher.¹⁰⁶ The latter regarded tumor regression as the result of an active host response that might reflect to some degree the normal growth pattern of the placenta during pregnancy. His concept of involution was one of direct strangulation, beginning with encapsulation of the embolus and ending with total fibrosis and isolation from the maternal circulation.

The question of whether spontaneous regression has ever led to a true recovery from choriocarcinoma has been debated. A number of cases were reviewed by Park and Lees³ in 1950, and we plan to evaluate the literature since that time in a later publication. It is possible that many reports of fortuitous involution reflected errors in diagnosis; nevertheless, a small percentage of cases has involved actual choriocarcinomatous tissue that regressed and was not succeeded at some later time by new growths. To our knowledge, the longest follow-up to date has been that of Brews.^{107, 108} In 1939, this investigator reported a 30-year-old woman who had had 5 normal deliveries. She hemorrhaged 12 days after her last childbirth, and tissue was noted in the vaginal orifice 24 days *post partum*. On the forty-first day, 2 vaginal metastases were found and shown to be choriocarcinoma on biopsy. The lungs were clear by X ray. One nodule was excised altogether, while the other, because of danger to the urethra, was left in the anterior wall. The uterus, tubes, and ovaries were removed. A week following surgery, the anterior mass had shrunk to one third its original size, and by 4 weeks was palpable but no longer visible. By the twenty-fourth day, nothing could be felt in the tissues. Brews had the opportunity to examine his patient once again in 1950, 11 years and 3 months after operation. There had been no recurrence of disease.

Whether deported benign trophoblast may proliferate and become neoplastic in an ectopic site has been argued endlessly. Among the earlier

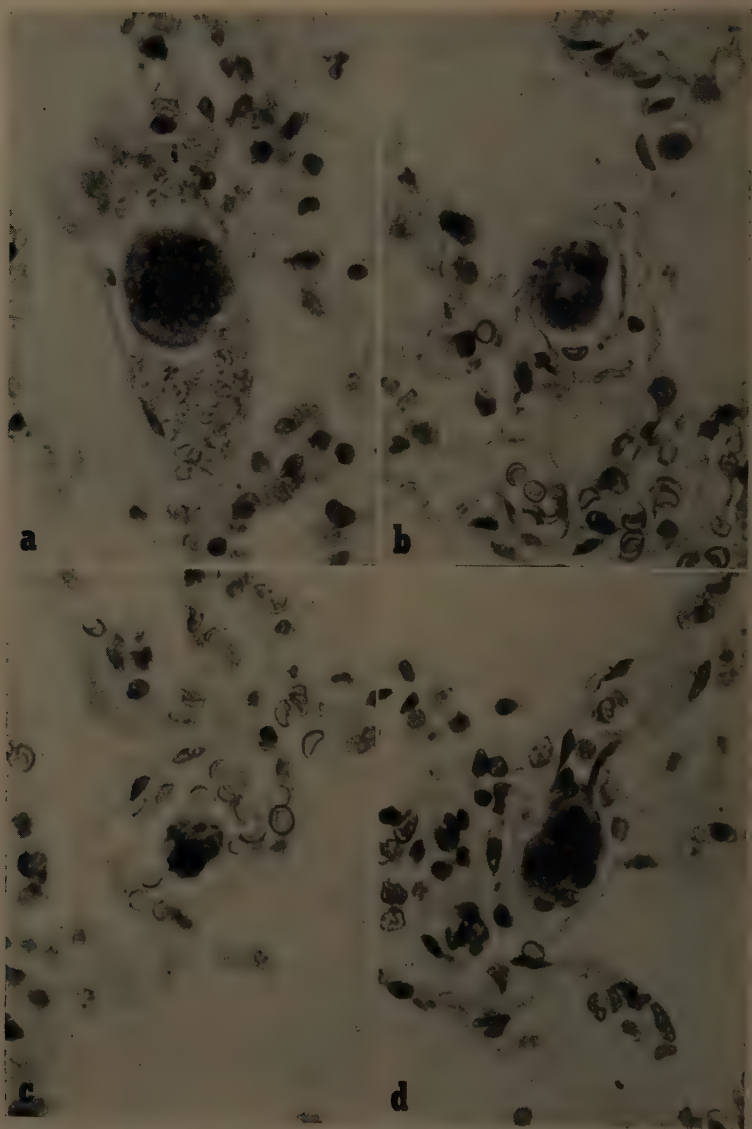


FIGURE 13. Spontaneous regression of deported syncytium. Successive morphologic stages in the degeneration of typical trophoblastic emboli, all photographed at same magnification. (a) Well-preserved syncytial mass in maternal lung. The patient, a 32-year-old Para V with a history of eclampsia following her first pregnancy, died with a 7½-month stillborn fetus and findings of coliform septicemia and toxic glomerulonephritis. Two sisters had died of renal disease during pregnancy. (b) Early degeneration with cytolysis and loss of nuclear detail. From same case as (a). (c) Karyorrhexis in advanced breakdown of tissue. Patient died with *post partum* surgical shock, pituitary hemorrhage and necrosis, and colloid carcinoma of the splenic flexure. (d) Rubbed-out appearance of deported syncytium in late stages of lysis, in 22-year-old Para I who died with toxic glomerulonephritis, pulmonary congestion and edema, and bronchopneumonia following the spontaneous delivery of a 7-month macerated fetus. From Bardawil *et al.*

reviewers, few have omitted at least a passing speculation about the origin of extrauterine choriocarcinoma, suggesting either that the tumor arose in formerly quiescent cells that underwent some sort of mutation, or that the deported tissue was malignant at the outset. The period of latency following known impregnation or delivery is usually brief and leaves little doubt of the source of trophoblastic tumor tissue, but there have been cases in which the question of teratoma deserves consideration. In 1935, Feiner¹⁰⁹ reported a case of fatal choriocarcinoma with pulmonary metastases in a 28-year-old

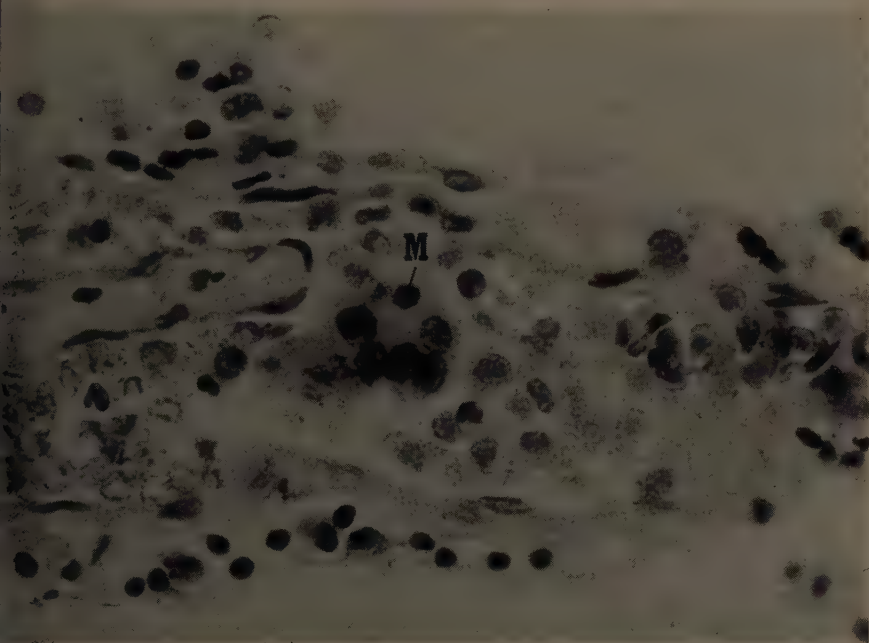


FIGURE 14. Deported syncytial embolus, showing at *M* a suggestive mitotic figure in prophase or early metaphase. From same case as in FIGURE 13 (*c*).

woman whose only known pregnancy had occurred at the age of 25 and had terminated in miscarriage after 4½ months. The period of latency in this patient was 2½ years. An even more striking case was described by White¹¹⁰ in 1955. The patient was a 59-year-old Para VIII, Gravida VIII, who had borne her last child at the age of 40. Her periods were regular until the age of 55, when a sudden hemorrhage occurred, was diagnosed as menopausal menorrhagia, and treated by irradiation of the pituitary and pelvis. Three years and 4 months after this artificial menopause, she returned with a pelvic tumor the size of a 14-week pregnancy and also vaginal bleeding. Total hysterectomy and bilateral salpingo-oophorectomy were performed. Six weeks after operation, she had 2 vaginal lesions and her lung films showed multiple metastatic masses. Despite irradiation, she died in the ninth post-operative week, and autopsy revealed tumor masses in the vagina, brain,

cerebellum, thyroid, spleen, kidney, lungs, and hilar nodes. It is difficult to decide where to fix the origin of the choriocarcinoma in this patient, and a good case may be made in favor of either the reawakening of dormant residual trophoblast from an earlier pregnancy, or true *de novo* teratoma.

Once transported to another site in the body, is a mass of syncytium likely to grow and become a tumor? Conclusive evidence of syncytial division is lacking, and most investigators consider the syntrophoblast an ontogenetic dead end that ordinarily exercises a well-delineated function and then is either cast aside at birth or biologically suppressed if it remains. Amoroso¹³ has shown a definite mitotic spindle apparently within the syntrophoblast of a rabbit placenta, but qualified his discovery as a possible cytotrophoblastic component. In the clinical series already summarized we have observed a mitotic figure in deported syncytium (FIGURE 14), apparently either prophase or early metaphase, but we must entertain similar reservations. It is of some interest that in the patient, a 27-year-old woman who died 2 days after delivery, a colloid carcinoma of the splenic flexure was found; although these two findings most probably bear no relation whatever to one another, this coincidence nevertheless deserves mention.

Immunological Sequelae

In addition to the onset of congestive failure following pulmonary obstruction by deported tissue, nonmechanical disturbances may result from the escape of cells or diffusible chemical agents into the maternal circulation should the localized trauma of trophoblastic or villous avulsion expose fetal connective tissue or blood vessels. Such denudation might be expected to furnish a ready avenue for the passage of fetal blood cells into the intervillous space. That erythrocytes may wander from fetus to mother in ordinary pregnancy and in delivery has been indicated by immunological experiments.^{111, 112} This finding, together with the morphologic demonstration that atypically shaped blood cells can travel from maternal to fetal vessels,¹¹³⁻¹¹⁵ supports the classic concept of autosensitization in erythroblastosis fetalis.

Certain disease states apparently due to hyperimmune reactions seem to be associated with pregnancy in one way or another. Ward and Gunther¹¹⁶ have described several cases in which disseminated lupus erythematosus arose in connection with normal and toxemic pregnancy. We have had the opportunity to study a case of scleroderma that became manifest during or immediately after an episode of eclampsia.¹¹⁷ Gutmacher¹¹⁸ in 1943 described a pregnancy complicated by scleroderma, but was unable to establish any definite relation between the two conditions. Although skin disorders are not specifically associated with pregnancy, there does appear to be some connection between gestation and the rare but transitory dermatological disturbance appropriately called herpes gestationis.¹¹⁹ Abdominal pruritis in pregnancy relieved by antihistaminics was reported by Kasdon¹²⁰ in 1953.

The degenerative changes occurring in the placenta during the course of pregnancy also merit consideration as a possible manifestation of hyper-

sensitivity. Montgomery¹²¹ believed the placental necrosis of gestation to be a physiological process initiated by the attack of a blood-borne "ferment" on the syncytium. In 1935, Tenney¹²² described a thickening of the villous basement membranes apparently specific in eclampsia and severe toxemia; this tissue change, an accelerated normal age change, was not seen in other gestational disorders. A year later, the same author¹²³ reported that the disintegration of syncytium, also a primary change in eclampsia in his view, correlated in extent with the clinical severity of disease. In addition to trophoblastic destruction or hyalinization, Tenney reported an apparent congestion of villous vessels in eclampsia;¹²⁴ one may wonder whether the latter effect might not represent local alteration in vascular permeability. Wislocki and Dempsey,¹⁹ in their histochemical analysis of eclamptic placentas, regarded premature aging and accumulation of phosphatases as the principal pathological features, with a concurrent decline of nuclear DNA in the syncytium and increase in density of collagen fibers. Gray *et al.*¹²⁵ have suggested that the presence of collagen plaques and fibrinous necrosis in the placenta of spontaneous abortion may imply some form of collagen disease, such as rheumatoid arthritis; serum from such patients was found to give positive latex flocculation tests in the majority of cases. The occurrence of so-called fibrinoid in the villi and in Nitabuch's layer may mean that the maternal-fetal boundaries actually function as vast fronts of antigen-antibody interplay and, perhaps, even as sites of direct antibody deposition. The absence of such matter in the decidua vera has led Amoroso¹²⁶ to wonder whether the layer of Nitabuch may not be dependent upon the continued presence of the fetus. Notions of fetal-maternal allergy are supported somewhat by the recent work of Paine,²⁰ who found that a fibrinous deposit accumulates slowly in the stroma of toxemic villi denuded of trophoblast. Possibly his observations represent a focal immunological precipitation within the ground substance, but a firm case for hypersensitivity cannot be established on this basis alone.

A significant rise in the count of circulating eosinophilic leukocytes has been described by Marcuse⁴⁹ in connection with trophoblastic deportation. This phenomenon, occasionally observed during normal pregnancy as well, was interpreted by Marcus and his colleagues¹²⁷ as a kind of poststress ACTH-withdrawal syndrome. Does eosinophilia in a pregnant woman indicate a systemic foreign body reaction against the contents of the uterus and the marshaling of cells for the synthesis of specific antibodies? At the present time no answer can be given, but the speculator may take heart in the contemporary investigations of Speirs,¹²⁸ assigning to the eosinophil a central role in host responses.

The hypergamma globulinemia demonstrated by Novell and de Haan¹²⁹ in 2 cases of severe pre-eclampsia, together with the rise in fetal salvage reported by Badawy and Gabrawy¹³⁰ following the treatment of threatened abortion with pyribenzamine, also point vaguely toward some sort of immune reaction. Once again, however, one can do little more than wonder. Evidence thus far has been only suggestive and has brought in its wake more problems than solutions.

THE PLACENTA IN IMMUNITY

Toxicity of Placental Antisera

In vivo. As early as 1886, Fehling¹³¹ had commented on the apparent association between pregnancy and chronic nephritis; in a group of 4 patients all of whom had shown albuminuria during gestation, he noted degenerative placental lesions, but was at a loss to offer any clear explanation for the tissue damage. When the monograph of Schmorl²⁵ appeared in 1893, with its suggestion of a direct connection between syncytial deportation and the appearance of clinical eclampsia, widespread interest was aroused in the possible role of placental tissues in allergic reactions. Shortly before Ehrlich had announced his so-called side-chain theory of antibody synthesis and this novel concept seemed uniquely appropriate in accounting for the picture of toxemia, particularly albuminuria.

Veit and Scholten,^{37, 42, 43} in the first major presentation of a unified theory of the pathogenesis of eclampsia, explained the *Schwangerschaftsnieren* or nephritic kidney seen in pregnancy as a consequence of deportation. In their view, deported trophoblast or its extracts stimulated the production of syncytiolysins by the maternal organism, leading in turn to the destruction of syncytial emboli. Ordinarily the maternal kidney was able to excrete the proteinaceous residua of syncytiolysis, and there were no clinical disturbances. This was the minimal albuminuria noted during pregnancy. In the event of massive deportation, however, the capacity of the mother to form syncytiolysin might be overwhelmed, lysin might not be elaborated rapidly enough to neutralize the toxicity of embolic placental tissue, and resultant damage to the kidney by circulating toxins would be manifest clinically in proteinuria.

Other investigators made numerous efforts to induce abortion or eclamptic convulsions in animals by the use of antisera against placenta. Techniques of immunization were crude at best, and asepsis was rarely sought. In the majority of experiments, raw saline suspensions of minced animal or human placenta were injected intravenously or directly into the peritoneal cavity. It is not surprising that results varied dramatically from one laboratory to another, some workers claiming the successful induction of abortion and/or convulsions, and others failure. In 1903, Dobrowolski¹³² reported that injection of placental antiserum in pregnant guinea pigs led to the precipitous delivery of stillborn or nonviable young. This contrasted with the negative results of similar experiments conducted in the preceding year by Ascoli.¹³³ Wormser¹³⁴ was able to induce delayed abortion in one animal, but obtained convulsions only when subdural injection of antiserum was accompanied by direct brain trauma. Weichardt,¹³⁵ treating rabbits with a mixture of placental suspension exposed to homologous antiserum, obtained lethal convulsions and evidence of widespread hemorrhagic necrosis in 3 animals, but negative results in 6 others; in a series of 10 attempts, Wormser and Labhardt¹³⁶ repeated his experiments, with no success. The latter also referred to about 23 failures by other workers.

In more recent years, Cohen and Nedzel¹³⁷ prepared a rabbit antiserum against guinea pig placenta, and injected this material in 6 pregnant guinea pigs. Five animals aborted within 1 to 5 days, bringing forth stillborn young, and the sixth was considered a probable abortion. Two controls injected with normal rabbit serum failed to abort. Histological findings included uterine spasm, flattening of syncytial cells, and eosinophilia, but no remarkable liver or kidney changes.

Similar findings were reported by Seegal and Loeb¹³⁸ in the same year. In a total of 68 pregnant rats injected with rabbit antiserum to rat placenta, two thirds of the fetuses were resorbed and widespread placental hyaline degeneration was noted. It is difficult to interpret these results, since the same pathology was later produced by the injection of rabbit antiserum to rat whole blood, and succeeding investigations with placental antiserum^{139, 140, 141} have failed to cause abortion in pregnant animals.

Of singular interest was the discovery by Seegal and Loeb¹⁴² in 1943 that antiserum prepared against homologous placenta was likely to produce in injected animals a chronic and often fatal nephritis. Their results were confirmed in later experiments.^{139-141, 143, 144} The observed histopathology was consistent with progressive glomerulonephritis, and showed classic lesions, including glomerular swelling, thickening of the basement membrane, crescent formation, and eventual obliteration of the tufts. The earlier observations of Cohen and Nedzel were thus contradicted.

The recent experiments of McCaughey,¹⁴⁵ using duck antisera to term rat placenta, have underscored the findings of Seegal and her colleagues. Evidence of glomerulonephritis was obtained within 5 days after injection, and placental antiserum did not appear to jeopardize the outcome of concurrent pregnancies. Baxter and Goodman,¹⁴⁶ in 1956, reported that renal medulla, lung, and placenta were as potent as renal glomerular tissue in stimulating the production of nephrotoxic serum. Other tissues were vastly inferior antigenic sources, or showed no activity.

The question of what function circulating antibody to placenta may serve in the pathogenesis of eclampsia has commanded the attention of many investigators since the time of Schmorl. Representative publications have been cited in the present review, and constitute only a small portion of the many reports dotting the German literature during the interval approximately between 1893 and 1905. In recent years the subject once again has been brought into focus, largely through increasing knowledge of so-called nephrotoxic antibodies. The work of McCaughey,¹⁴⁵ who openly suggested a direct relation between the glomerulonephritis induced by placental antiserum and similar lesions seen in pregnancy, has been mentioned. It is appropriate to cite also the report of Lin,¹⁴⁷ who gave 10 virgin female rats repeated intraperitoneal injections of term rat placental suspension, and then mated the animals. She found increased blood pressure, albuminuria, and arteriolosclerotic changes especially prominent in the renal glomeruli. Although all animals carried to term, two had stillbirths and one convulsions. Lin wondered whether these findings might not imply allergy to placental protein as a basis for toxemia.

On the strength of the apparent effects of placental antisera, it is possible to chart a pathogenetic route that could lead to toxemia or frank eclampsia. The sequence is outlined broadly in FIGURE 15, which is presented as an example of the type of speculation that can be stimulated by our present knowledge of placental immunity. Soluble and diffusible antigenic substances from any conceivable site in the placenta or fetal tissues might find their way into the maternal circulation and thence to appropriate sites for antibody synthesis. The deportation of trophoblasts and the impaction of embolic tissue fragments in various maternal organs offer an additional mechanism of antigenic stimulation. Once elaborated

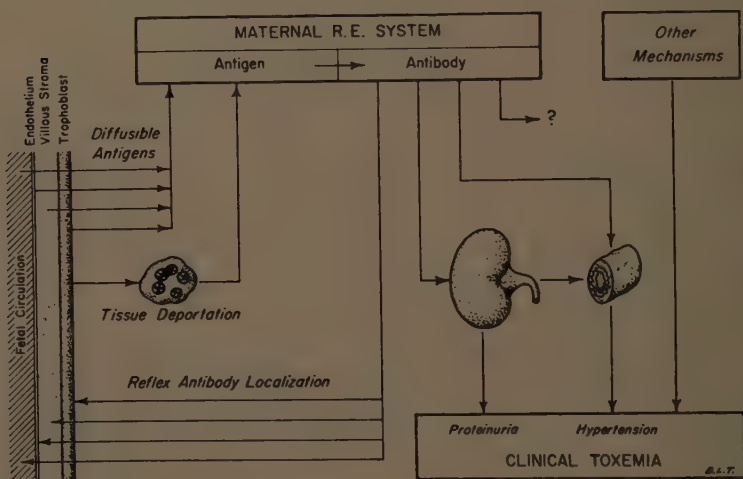


FIGURE 15. Pathways of hypothetical autosensitization associated with normal pregnancy or trophoblastic tumor. Diffusible agents from either fetus or placenta, or whole tissue fragments in the case of deportation, are carried by the maternal circulation to distant sites appropriate for antibody synthesis. Antibodies in turn may react with the sensitizing antigen *in situ* or within the blood stream. Immune reaction may occur also with renal and vascular elements, eliciting the clinical syndrome recognized superficially as toxemia, and perhaps other disturbances such as those of the collagen disease groups.

circulating antibodies might be carried back to the uterine lumen and react with corresponding antigens in the fetus or placenta. The question of haptenic formation remains open and cannot be answered. In the event that similar materials occur in the maternal tissues, cross reactions will become probable, and thus one might account for the renal injuries noted in eclampsia. Hypertension, a well-known feature of the syndrome, might be due either to renal damage or to a direct effect upon vessels themselves. One may postulate other forms of pathology, but with the understanding that such notions are completely conjectural. It must be borne in mind that the clinical picture called eclampsia may be a type of final common pathway in the sense that a number of concurrent though unrelated processes may contribute to the establishment of a single syndrome, just as innumerable

forms of physical and emotional trauma may cause the over-all reaction designated a stress response.

In vitro. At the same time that poorly defined syncytial toxins were indicted as a primary factor in the genesis of eclampsia, many workers tried to produce unequivocal cytolysis of placental tissue, or definite precipitation reactions, between fragments of villi and serum from eclamptic patients. Some, like Weichardt,¹³⁵ Kawasoye,¹⁴⁸ and Zironi,¹⁴⁹ claimed specific precipitation reactions; others, including Dobrowolski,¹³² Wormser,¹³⁴ and Cronson,¹⁵⁰ reported negative results. These early experiments involved both test-tube and hanging-drop preparations and were described in terse and vague terms. There does not appear to have been anything resembling a consensus.

Several workers^{136, 151, 152} at this time suggested that syncytiolysins or similar substances in the maternal blood stream might provide the substrate for an *in vitro* serologic test for the diagnosis of early pregnancy. Even though this method was proposed with enthusiasm, we are not aware of any published series that might confirm or refute its practicability. Indeed, no immunological test for pregnancy, regardless of the antigen employed, has ever earned universal acceptance.

Cohen and Nedzel¹³⁷ used the precipitin technique in checking the development of placental antiserum in rabbits. They also claimed positive reactions between full-term placental protein and serum from two patients with a history of habitual abortion. Gray *et al.*,¹²⁵ working with serum from cases of spontaneous abortion and using placental suspensions as testing antigens, have reported a significant correlation between visible villous lesions and positive latex-flocculation titers.

A direct approach has been undertaken by Bassett and his co-workers.¹⁵³ Rabbits were immunized against washed and homogenized human placenta, and portions of the resultant antiserum incubated with tissue cultures of human skin. The media containing antiserum exerted a selective inhibitory effect against fibroblastlike cells, with rounding and lysis, but there seemed to be no apparent toxicity to epithelioid elements. This result is difficult to interpret in terms of placental allergy, since a mixed antigenic stimulus was employed.

Antigenic Localization in the Placenta

Although it is certain that placenta contains substances capable of eliciting antibody formation, there has been no satisfactory demonstration of their nature or histological distribution. The cross reactivity with kidney and nephrotoxicity of placental antisera suggests the existence of a common antigen, and its situation within the renal parenchyma might be expected to offer some information as to its probable chemical structure. The experiments described in the preceding sections indicate that the nephritis produced with antiserum against placenta is well localized in the glomeruli. This finding was reinforced by Greenspon and Krakower¹⁵⁴ in 1950, when they isolated entire glomeruli from dog kidney and demonstrated that these bodies appeared to contain nephrotoxic antigenicity to a far greater degree

than other tissues of the organ. In 1953, Cruickshank and Hill¹⁵⁵ showed binding by a fluorescent conjugate of antiglomerulus serum in the basement membrane, seemingly within the reticulum. The investigations of Mellor and his colleagues,¹⁵⁶ also employing the fluorescent antibody technique, have added weight to the supposition that the nephrotoxic antigen occurs in the glomerular basement membrane. Goodman *et al.*¹⁵⁷ in 1955 used serologic agglutination and absorption tests to buttress their earlier findings; in a very recent communication, Krakower and Greenspon¹⁵⁸ have reported that



FIGURE 16. Placental villus stained for gamma globulin by the fluorescent antibody technique, showing the accumulation of protein in a syncytial bud. C, cell column; K, syncytial knot; S, stroma of villus; T, trophoblast. From 35-mm. color transparency. Reproduced from Bardawil *et al.*¹⁶¹

although the nephrotoxic antigen appears most richly concentrated in the glomeruli, it can be found universally in capillary basement membranes of other organs. In perspective, one is left with the suspicion that the principal immunological activity of placenta may, in fact, reside in vascular or subtrophoblastic basement membrane, and perhaps not at all in the chorionic epithelium.

In a somewhat different approach, Pressman and Korngold¹⁵⁹ have prepared radio-labeled rabbit antirat placenta globulin and injected it intravenously in virgin and pregnant rats. Localization was determined on the basis of measurable tissue radioactivity. In virgin animals, globulin was bound chiefly in the liver and kidneys, while in the pregnant rats there was

localization in these sites and also in the placenta. In the case of anti-kidney serum, protein was retained in the same tissues but, to a lesser degree, in placenta. The investigators concluded that abortion and nephritis may be due to separate antibodies, a judgment certainly acceptable if one bears in mind the broad mixture of antigens anticipated when a gross tissue rather than an isolated compound is employed as a stimulus.

In an earlier study performed in our laboratory,¹⁶⁰ the fluorescent-tagging method was used to seek circulating antibodies against placental components in eclampsia. Serum globulin from patients in pre-eclampsia, acute eclampsia, and the convalescent phase were conjugated to fluorescein isocyanate and applied as a stain against normal and toxemic placental sections. This investigation has failed to demonstrate any free antibody capable of localization in the villi or decidua in either normal or eclamptic women. Because of the vagaries of the technique, as well as the fact that a totally unknown immunological system was involved, only a tentatively negative result can be reported. If circulating antibodies do exist in eclampsia, a more sophisticated approach may be necessary to demonstrate them. Surveys of the distribution of gamma globulin in normal and pathological human placentas¹⁶¹ have revealed a disposition attributable to passive diffusion from the maternal and fetal blood vessels. Essentially no globulin has been observed in the trophoblast, except in scattered areas of syncytial density such as the knots (FIGURE 16). Here localization was intense. One may wonder whether such an accumulation of serum globulin might not furnish the climate favoring immunological reaction by virtue of prolonged chemical contact.

Nature of Placental Antigens

Understanding of the nature of whatever circulating antigens or antibodies may be peculiar to pregnancy has been equally primitive. It is possible to point in several ill-defined directions in which initial forays have been undertaken and which appear promising even at current levels of information.

In a provocative pilot study, Thorns and his colleagues¹⁶² at the Boston Lying-in Hospital have isolated a so-called specific substance in serum from pregnant women. After immunizing rabbits against pooled pregnancy serum, they absorbed the resultant antiserum against nonpregnant human serum, and then tested the product for immunological specificity by gel-diffusion analysis. This mode of attack has revealed the presence of one, and possibly two, such specific substances that might function as either antigens or antibodies and appear to exist in the serum only during pregnancy.

A similar technical approach has been used in the laboratory of the authors. Rabbit antiserum prepared against a suspension of washed and perfused human umbilical cord has shown the presence of at least three probable antibodies. In gel-diffusion plates, these substances yield definite precipitation lines when tested against extracts of both cord and placenta, but exhibit no affinity for fetal blood cells (FIGURE 17). The continuity of lines suggests that these tissues may contain antigenic materials in common, but unfortunately the nature of the latter has not been deduced.

The extant literature contains few contributions to aid the investigator in learning the chemical properties of placental antigens. Cohen and Nedzel¹³⁷ had assumed the sensitizing stimulus in their own series of immunizations to be a placental tissue protein, but did not isolate it. Smith and his co-workers¹⁶³ claimed the discovery of a noxious protein element in the sera of toxemic patients; both the existence and essence of this bizarre material remain in question, however. An apparently characteristic globulin has been isolated from placentas of toxemic patients; Lapan and Shey¹⁶⁴ found that the protein extract gave significantly positive skin-test reactions in pre-eclamptic patients. One may note with considerable interest that this very same globulin fraction also elicited positive cutaneous responses

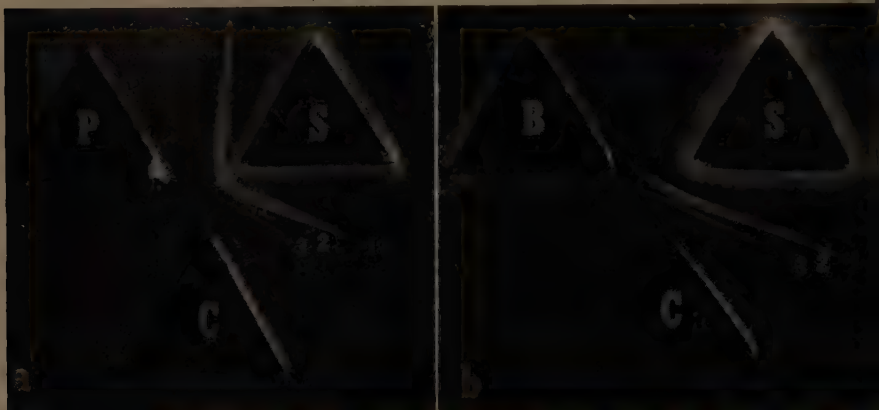


FIGURE 17. Immunological characterization of antigens in placenta and umbilical cord, by means of agar-gel diffusion technique. 'B', fetal red blood cells; C, umbilical cord; P, human placenta; S, rabbit antiserum to normal term human umbilical cord. (a) Apparent common antigenicity of gross placenta and umbilical cord. (b) Exclusion of erythrocyte antigens from precipitation system.

in individuals with chronic nephritis, and thus ponder another hint of cross-antigenicity between placenta and kidney.

Novell and de Haan,¹²⁹ who demonstrated an increase in serum alpha- and beta-globulins in pre-eclamptic patients, have suggested that these elevated glycoproteins may represent the true antigens active in eclampsia. Although the agar immunoelectrophoresis experiments of Scanu *et al.*¹⁶⁵ indicate that the protein moieties of normal alpha- and beta-lipoproteins are both active and distinct antigenically, there is insufficient evidence at hand to permit exclusion of other possibilities.

In the realm of placental antigens, greatest favor is probably due the carbohydrates. It was the opinion of Gray *et al.*¹²⁵ that the substance responsible for their agglutinations in spontaneous abortion cases was most probably a mucopolysaccharide. Their conjecture derives support from the earlier work of Kaku¹⁶⁶ in 1953. The latter, in perhaps the most important investigation yet undertaken to elucidate the immunoanatomy of the placenta, showed that both human and rabbit placental polysaccharides

could elicit specific antibodies. Lipid components were inactive. Such extracts, moreover, reacted with approximately one quarter of toxemic samples tested, failed to respond with controls, and were capable of causing liver and kidney damage in pregnant rabbits previously injected with toxemic serum.

It will be recalled that the evaluation of gross placental antisera by histochemical tracer techniques has shown a localization in the glomerular basement membrane of the kidney. Whether placental and renal nephrotoxic antigens are one and the same is unknown, and the fact cannot be established by morphologic evidence alone. The recent work of Milazzo,¹⁶⁷ however, offers at least a partial answer to the question of chemical identity. Reticulin was isolated by trypsinization from human and rat renal glomeruli and used to prepare specific rabbit antisera. After rats were injected with the product, a search was made for renal lesions. The material was found to contain a stable noncollagenous antigen that was not inactivated by various salt and buffer solutions except in extreme pH ranges. Milazzo identified the active antigen as a lipoglycoprotein. Clearly one is led to wonder what relationship, if any, there may be between the antigen of Milazzo in the kidney, and that of Kaku in the placenta. Indeed, it is quite possible that the two have nothing to do with one another chemically, but may be merely dwellers in the same mirage.

A BIOLOGICAL CONCEPT OF CHORIOCARCINOMA

Spontaneous Regression: Fact or Artifact?

Willis,¹⁶⁸ in his classic monograph on tumor metastasis, observed that even the most malignant of neoplasms are nevertheless products of the host tissues and therefore wholly integrated elements of a biological community. As such, they possess a finite life span, just as do normal tissues of the body, and therefore what occurs to both tumor and host depends essentially upon the intrinsic growth potential of the cancer and upon the manner in which its nutritive and other requirements are gratified. The situation in which the effective life span of a neoplasm is less than that of its host may be defined as spontaneous regression.

Published accounts of apparently gratuitous involution of tumors have been legion. Whether such reports have been genuine or spurious remains another matter. Gaylord and Clowes,¹⁶⁹ noting that the spontaneous cure rate in mice appeared to be roughly 23 per cent, suggested that spontaneous regression of human growths might be a common event. In her analysis of 53 cases, Frauchiger¹⁷⁰ proposed the following criteria for spontaneous regression: (1) concurrent histological and clinical diagnoses; (2) known course and termination of a case; and (3) microscopic evidence of regression. It was her opinion that no case had satisfied all of these requirements and that spontaneous regression must be dismissed as merely a theoretical possibility. Rohdenburg¹⁷¹ and Strauss¹⁷² attempted to catalogue the various situations in which spontaneous degeneration might occur; more recently, Everson and Cole¹⁷³ have offered a list of possible etiologic factors in

spontaneous regression of cancer. The latter authors, after a review of 600 reported cases, rejected all but 47 as insufficiently documented; indeed, they declined to consider any report prior to 1900 on the basis of probable misdiagnosis. Diagnostic error in the evaluation of malignancy and regression appears to have been recognized¹⁷⁴ as early as 1852 and has been mentioned in the introduction of the present paper as a significant source of interpretive confusion.

The apparently spontaneous regression of choriocarcinoma has been described sufficiently often to have become accepted as an occasional if not frequent phenomenon. Very rarely, teratomata of the testis has been known to involute for no demonstrable cause, as in the famous case of Prym.⁷⁵ During the decade following the work of Marchand there were numerous accounts of seemingly ectopic choriocarcinomata with no visible intrauterine locus. From time to time, metastatic lesions have appeared to degenerate and vanish after a primary growth was excised. Some situations have been quite bizarre; de Zalka¹⁷⁵ in 1928 reported 2 cases of choriocarcinoma with apparent primary sites in ovary and liver, but no uterine disease whatever, and he raised the question of a retrograde venous metastasis of chorionic cells. Park and Lees³ have summarized the literature up to 1950; although a number of case reports have reached print since that time, a detailed consideration of individual publications is beyond the intent of the present paper. It must be asserted, however, that radiological evidence of metastatic regression is not *per se* specific; histological verification of regression has been the exception and not the rule, and the generally irregular following of cases has relied upon sporadic gonadotropin assays for evidence of tumor involution. All in all, the critical investigator would be justified in regarding a meticulously studied and documented example of spontaneous regression in choriocarcinoma as the rarest of cases.

There has never been a wholly satisfactory explanation for the few patently genuine cases in which histologically established choriocarcinomata have been proved to degenerate gratuitously. Apart from the peculiar genetic constitution of this tumor, there is no palpable guarantee that the idiopathic involution of such a growth differs intrinsically from that of any other regressed tumor, for example, a retinoblastoma. Some hypothetical causes for recession have savored more of the red stone of the alchemist than of the histologist's microscope. Thus Peters,¹⁷⁶ in 1902, blamed the proliferation of deported trophoblast upon an unchecked "abnormal dignity" (*sic*) of the tissue, and von Velits,¹⁷⁷ three years later, mused that the involution of both placental and ectopic trophoblast was due to the ubiquitous *vis medicatrix*.

The earlier work of Veit has led Anspach and Hoffman¹⁷⁸ to assume that spontaneous cures are due to the elaboration by the host of a syncytiotrophoblastin that inhibits and finally destroys the trophoblast. Schmauch¹⁷⁹ and Frankl¹⁸⁰ have followed similar paths of reasoning. Mickulicz-Radecki¹⁸¹ wondered whether regression might not be due to direct damage by hemorrhage. The observations of Risel¹⁰⁵ and Teacher,¹⁰⁶ suggesting the picture of a granulomatoid response, have been described. If there has been any

consonance of opinion at all, it has tended in the direction of some kind of immunological reaction, as yet more fable than fact.

The Tumor and its Host

The placenta and fetus occupy a unique position in the genetic cosmos of the mother, since they arise in part from her own tissues and, in part, from those of the father. Thus, every pregnant woman carries within her uterus a genotypically distinct biological entity and, hence, a potential if not actual foreign body. It is reasonable to wonder whether fetal or placental constituents, whether normal or pathological, might not function as a sustained antigenic stimulus, leading sooner or later to the production of one or more antibodies in the maternal reticuloendothelial system. Reflex localization of such antibodies, syncytiolysins, or whatever one will might be expressed in a variety of disease syndromes attributable to autosensitization (FIGURES 15 and 18).

That such disorders occur is commonly accepted, and one need do no more than mention erythroblastosis fetalis as the archetype of the group. Allergies to one's own nervous tissue or lens protein are experimental accomplishments of long standing. Witebsky and his colleagues, working with rabbits, have shown that thyroid autoantibodies can be produced by standard methods¹⁸² and have suggested that an autosensitization may play a direct role in the development of chronic thyroiditis;¹⁸³ in the human being, Roitt and his co-workers¹⁸⁴ have showed the presence of autoantibodies to thyroglobulin in Hashimoto's disease. The criteria recommended by Witebsky¹⁸³ for the establishment of autoimmunity as the mechanism of a pathological process might be applied conveniently and logically to the problem of spontaneous regression. Freund *et al.*,¹⁸⁵ in 1953, showed that autosensitization to guinea pig testis or sperm may lead to failure of spermatogenesis, seemingly through destruction of the spermatogonia. The phenomenon has been found to be organ-specific but not species-specific by Voisin *et al.*¹⁸⁶ That this kind of allergy may be a significant factor in human sterility has been suggested by Wilson,¹⁸⁷ who found agglutinins for sperm in the semen and blood of two sterile males. Indeed Blakely,¹⁸⁸ in 1937, had attempted to predict fetal sex *in utero* by serologic tests based upon a supposed maternal allergy to semen or sperm. In another area there has been evidence that the so-called collagen diseases, in whole or in part, may have to do with some kind of hypersensitization to nucleoprotein.¹¹⁷ Clearly, *horror auto-toxicus* is no longer an inflexible rule.

In recent years de Ruyck¹⁸⁹ has claimed the isolation of viruslike particles from choriocarcinoma and has taken his finding as evidence that this tumor may be of viral origin. Teacher,¹⁹⁰ in 1903, had described curious intracellular inclusions in trophoblast, and similar effects have been observed in our own material (FIGURE 12). Certainly, a viral theory would fit neatly into schemes to explain spontaneous regression, but the concept has not been accorded general acceptance, and the present authors are inclined to write off such apparent inclusions as probable artifacts of staining or degeneration. The question of whether placental protein may act upon the maternal

organism as a homologous or heterologous protein had been raised by Liepmann¹⁹¹ as early as 1905 and, thirty years later, Frankl¹⁸⁰ restated the problem, pointing out that the chorionic epithelium, stemming in part from the male germ plasm, might function as a foreign body as far as the mother was concerned.

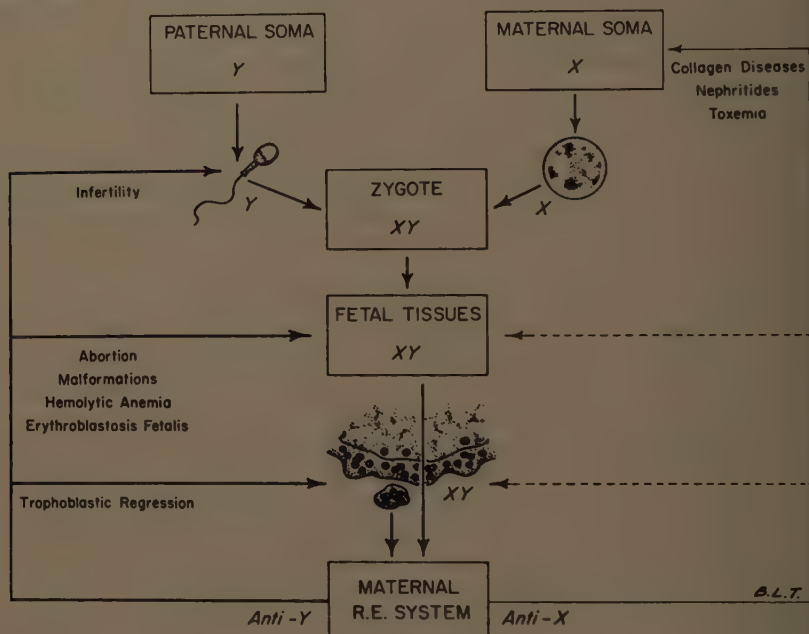


FIGURE 18. Fetus and placenta as homotransplants in the maternal organism. A speculative immunological pattern based upon the relevant genetic relations. Fertilization yields a zygote with specific surface antigens reflecting both maternal and paternal contributions. Thus the fetus and placenta constitute a tissue phase which is in part autologous and in part homologous with respect to its host. Escape of tissue particles or soluble extracts into the maternal blood stream, as suggested in FIGURE 15, may stimulate the synthesis of antibodies directed against the sensitizing antigens. The latter, in all probability, would consist of phenotypic expressions upon the cell surface, or cellular secretions, corresponding to genetic loci peculiar to the male chromosomes. Depending upon the site of interaction between antigen and antibody, numerous clinical syndromes may be imagined, and are listed in the diagram. In such a schema, it is possible to conceive of reflex injury to the maternal somatic tissues as well. The letters X and Y represent only classes of possible antigens peculiar to the female and male germ cells, respectively, and do not denote the so-called sex chromosomes per se.

In an attempt to set the spontaneous regression of choriocarcinoma into some kind of perspective, Schopper and Pliess¹⁹² in 1949 brought attention to two possible regulatory mechanisms. The one, a systemic factor, was essentially immunological and probably a circulating antibody elaborated in the maternal reticuloendothelial system, functioning to rid the mother's vascular and lymphatic channels of embolic trophoblast; the other factor was purely local, a kind of antitryptic agent formed by the decidua at the

fetal-maternal boundary and manifest in the laying down of a fibrinoid barrier against the advancing chorionic epithelium. The major contribution of these authors was the suggestion that choriocarcinoma might be analyzed as if it were an inoculation tumor of the type transplanted between experimental animals, because of (1) the way in which the ovum became implanted, (2) its apparent prior proliferative potential, (3) the possibility of spontaneous inversion in a suitable immunological climate, and (4) the fact that differences observed between indigenous neoplasms and inoculation tumors corresponded to those noted between ordinary cancer and choriocarcinoma. In short, to Schopper and Pliess is due credit for the first coherent assertion that a choriocarcinoma was not merely another local disturbance of cellular growth patterns, but rather a biological phenomenon reflecting a specific interaction between maternal and fetal tissues.

In 1952 the inoculation tumor concept was reiterated by Schuster,¹⁹³ who observed that the few cases of spontaneous regression on record involved only true choriocarcinomata, which were half foreign in essence, and never teratogenous choriocarcinoma in either male or female patients, the latter growths being entirely autologous in origin. Noting the seemingly short latent period preceding the appearance of a chorionic tumor, Schuster raised the question of viral induction, one that has been reviewed and dismissed previously in this paper. Schopper,¹⁹⁴ writing in the same year, underlined the apparent importance of the decidua in resisting the local invasion of trophoblast, emphasizing that a choriocarcinoma is a tumor *sui generis* and must be evaluated according to its own behavioral rules.

Dowling,¹⁹⁵ in 1957, restated the over-all problem, calling choriocarcinoma a frank homotransplant dependent for its very survival upon genetic identity between parent and fetus and a sufficient degree of dedifferentiation of the tumor. The fatal metastasizing growths, in his view, were extremely dedifferentiated. Malignant moles that regressed or remained locally confined were considered too highly differentiated to survive in the homologous maternal host.

Is a choriocarcinoma, then, a wholly independent tissue mass, perhaps with an autonomous growth potential all its own in much the same sense as implied by Greene¹⁹⁶ and reliant upon its specific interaction with a genotypically foreign host in the determination of its clinical destiny? Or may this strange tumor play a somewhat more subservient role in the maternal tissues, with dependency upon unknown endocrine or other little understood systemic agents? The equivocal results of hormonal therapy, well summarized in the scholarly study of Smalbraak,¹⁹⁷ are not likely to grace any currently known humoral factor with the power of controlling the growth of pathological trophoblast. Pearson and his colleagues,¹⁹⁸ in 1956, could reach no definite opinion concerning the value of hypophysectomy in treating five cases of choriocarcinoma, and were certain that it accomplished nothing in two male patients with embryonal carcinomata of the testis. At the present time it may be better to consider choriocarcinoma as a discrete phenomenon and attempt to explore its interactions, if any, with the maternal soma.

Might the spontaneous regression of a choriocarcinoma be due to a form of feed-back effect, whereby the tumor produces its own inhibitors? Weiss¹⁹⁹ has suggested that growing tissues may elaborate diffusible substances that accumulate in the over-all body pool and reach a critical equilibrium concentration tending to brake further proliferation. If such a mechanism indeed exists, one may conceive of a dynamic balance between normal trophoblast and its own products, leading to choriocarcinoma when the scales are somehow upset. This and similar processes have been reviewed by Rose,²⁰⁰ who has discussed in particular recent findings that growth and differentiation may be abetted by nucleoprotein extracts of various organs but retarded by protein derivatives. Whether such protein extracts have any kind of immunological properties is totally unknown. One may cite in addition the work of Helff,²⁰¹ who has demonstrated the power of extracts of histolyzing larval tail skin to cause the involution of mouse mammary tumors; a cross-species effect of this sort argues in favor of an intrinsic growth inhibition factor, but little more can be said on the subject.

Apart from direct cytotoxic manifestations, the presence of antibodies appears to exert definite but poorly comprehended effects upon growing tissues. Such phenomena, in their more general aspects, have been reviewed by Nace.²⁰² Sturtevant²⁰³ and Emerson²⁰⁴ have pointed out that under special circumstances an antibody may be able to induce a genetic mutation. In brief, a genetically determined cellular antigen might stimulate the production of a circulating autoantibody that, in turn, might combine with the original gene and thus, so to speak, cause a superficial alteration of the genetic template. In the case of a tumor such as choriocarcinoma, the antigenic change responsible for a "spontaneous" regression might occur in the microsomes, shown by Malmgren²⁰⁵ to possess cytotoxic antigenicity.

Additional results of induced or fortuitous mutation may be envisioned without difficulty. For example, a chromosomal alteration might lead to the loss of one or more of the so-called histocompatibility genes, extensively studied by Snell^{206, 207} and demonstrated by him to control antigens that play vital roles in the take or rejection of a transplanted tumor. The studies of Snell have been a fortunate consequence of the classic discovery by Gorer,²⁰⁸ in 1937, that tissue compatibility might be influenced by host reactions to gene-determined cellular antigens.

The frequent occurrence of chromosomal multiplication in malignancies especially to the tetraploid number, is commonly known. It is not surprising, therefore, that such changes appear to be related to the specificity of transplantation reactions and thus may constitute another variable in the evaluation of regressive phenomena. Hauschka and Levan^{209, 210} have found that, while diploid tumor transplants are generally strain-specific and tend to regress when implanted in unrelated genetic lines of animals, aneuploid neoplasms with chromosome numbers approaching a tetraploid model show sharply decreased specificity, and clinical malignancy. In a series of immunological experiments with mouse ascites tumors, Amos²¹¹ found diploid forms, as a rule, highly antigenic and immunologically active, while aneuploid tissues seemed much less potent. Levan²¹² has wondered whether

The apparent ability of tetraploid tumors to tolerate substantial variations in chromosomal morphology may not reflect gradual genotypic changes making the tumor stemlines increasingly efficient, and, one may add, probably better able to survive the vicissitudes of a foreign environment such as a secondary host. It is almost tautological to add that a careful study of the relationship between cellular ploidy and clinical malignancy in choriocarcinoma would be welcomed warmly.

The loss of transplantation specificity may be due to a process of antigenic simplification in which, as Gorer²¹³ has conjectured, one or two antigens might increase in quantity and gradually crowd out others. Gorer doubted that changes in specificity were caused by mutational shifts between dominant and recessive alleles, although he granted that there might be a high mutation rate for histocompatibility genes in mice. As possible causes for antigenic simplification, Hauschka and Schultz²¹⁴ have suggested (1) point mutations in the histocompatibility or H-loci (2) physical loss of part or all of chromosomal segments bearing H-loci, (3) loss or duplication of antigenically determinant chromosomes, and (4) variations in ploidy. According to a recent theory presented by Feldman and Sachs,²¹⁵ successful homo-transplantability among tumors may be caused by an increased resistance to isoimmune responses; adapted tumors might show a higher rate of antigen synthesis and thereby neutralize circulating cytotoxins produced by the host. It is also possible, as Kay²¹⁶ has observed, that malignancy may not depend so much upon a loss of specific antigens, or even increased synthesis as hypothesized by Feldman and Sachs, but rather upon some undefined change in the cellular surface which tends to mask their expression. One may wonder with Dowling¹⁹⁵ whether in a state of adaptation the host does not become a selective culture medium for those tumor cells least vulnerable to allergic reaction.

The heterochromatin of the sex chromosomes may also participate in the government of host-tumor interrelations. In 1955, Eichwald and Silmsker,²¹⁷ in a series of 149 grafts to related backcrosses in mice, noted that whereas most trials were successful, grafts failed regularly when attempted between male donors and female recipients, but might take in the reverse direction. The pertinent factor(s) has been found in all normal male mouse tissues examined, but only in one sixth of male tumors tested.²¹⁸ Is this possibly another expression of antigenic simplification? By way of explanation, Hauschka²¹⁹ speculated that male skin may require for sustenance androgenic hormones not available in the female recipient, and/or that female XX recipients, lacking the Y antigen, may form isoantibodies against the male XY grafts. The experiments of Peer,²²⁰ showing that skin transplants from father to offspring of either sex invariably fail, but that maternal grafts may survive for prolonged periods, establish the fact that the Eichwald-Silmsker phenomenon may occur in man as well as mouse, and raise obvious questions with regard to pregnancy. Why male grafts should be rejected is not understood beyond the pale of guesswork; a specific difference in antigenic pattern is very likely, and is implied by the discovery of Schultz²²¹ that the presence of a Y chromosome in an oocyte appears to cause the

synthesis of ribonucleic acid with a different array of bases than that observed in its absence.

One may ask whether a choriocarcinoma will go on to a fatal end if its chromosomal structure be female, and regress if it contains a male sex chromatin picture. Unhappily, the scant evidence now at hand is hardly gratifying. In 1955 Levij²²² noted substantial percentages of sex chromatin in 9 of 18 testicular teratomata, uniformly in 20 ovarian teratomata, and in a single placenta associated with a female infant. Two years later Oosterhuis and Levij²²³ applied the same method to 5 cases of choriocarcinoma in female patients; in 4 of the cases, the tumor sex chromatin corresponded to that of the child, showing a direct connection between such neoplasms in the female and the products of a concurrent or prior pregnancy. Two of the patients had borne male infants. Of 7 choriocarcinomata in males, 3 showed a female nuclear structure, implying that these growths were probably teratomatous. It would be instructive to examine the sex chromatin of choriocarcinomata shown to have undergone spontaneous regression, and one may hope that this remarkable technique may be applied more extensively in the future.

For convenience in genetic analysis, one may regard the fetus and placenta as constituting a kind of F_1 hybrid backcross homotransplant within the maternal uterus. Dowling¹⁹⁵ has compared this situation with experimental parabiosis, in which tumor susceptibility has been known to increase. Perhaps the survival of the placenta during pregnancy depends in some way upon the establishment of a state of tolerance in the sense defined by Billingham *et al.*²²⁴ Can intrauterine exposure to some unknown factor condition a female fetus for the eventual carriage of her own placenta and, if so, why does the same mechanism not apply to teratomata in the female? In the latter instance, does the autologous nature of the tissue exempt it from an immunological regulation that might otherwise restrain its growth and cause its ejection at term? One may ask also whether embryonal antigenicity, a possible determinant of the fate of the implantus, might not depend in turn upon the cytological maturity of the fetal tissues, failing to stimulate the host until a specific ontogenetic level has been achieved.

One of the most striking aspects of spontaneous regression in choriocarcinoma has been the apparent disappearance of metastases following the excision of a primary tumor. Aside from the obvious question of whether secondary growths behave as dependent satellites, one may suppose a constant and dynamic balance between the host defense mechanisms and the total amount of neoplastic tissue present in the body. Zeidman *et al.*,²²⁵ in 1950, found that when mice were injected with suspensions of transplantable homologous tumors, the number of metastatic takes was roughly proportional, up to a limit, to the number of viable cells inoculated. It is equally possible, however, that the rate of take depends more upon the quantity of cells per se than upon any effect attributable to the host, that is, that less tumor emboli survive simply because less are injected at the outset.

The survival of a choriocarcinoma in a patient may be the result of a prior conditioning of the maternal soma by normal trophoblast. In 1907, Flexner

and Jobling²²⁶ showed that the inoculation of rats with heat-killed tumor tissue appeared to enhance the proliferation of subsequently implanted viable fragments of the same growth. In mice, Kaliss and Molomut²²⁷ found that injection of antiserum to mouse tissues led to a seemingly complete failure of resistance to tumor homografts that exhibited little or no growth in normal control animals. In a recent review, Kaliss and Bryant²²⁸ have attributed such immunological enhancement to an induced alteration that allows the homograft to survive despite active host response. The effect has been compared with other possibly related reactions, including immunological paralysis by pretreatment with bacteria or their extracts.²⁰⁷ One may wonder whether, indeed, the showers of syncytial trophoblast that become deported to the lungs during a normal pregnancy might not serve a similar end by enhancing the growth of a later chorionic tumor.

Clinical Correlations

Diagnosis. The histological appearance of host reactions against trophoblastic metastases has been described in earlier sections. In addition to the central necrosis commonly observed in degenerating tumor masses, one may often see a peripheral breakdown in nodules of choriocarcinoma, raising the possibility of direct attack by some humoral factor, such as an antibody. At times this type of necrosis may confuse the investigator and cause a partially destroyed trophoblastic mass to be taken for an infarcted leiomyoma. One of us (W.A.B.) can recall having been so deceived on a past occasion.

Therapy. The study of a disease is in itself only an academic exercise. An excursion into oncology must eventually return to the question of what has been learned that may be applied to the treatment of patients; therefore, it is appropriate to conclude the present review of the vagaries of choriocarcinoma by considering what immunological investigations have contributed to our ability to aid those afflicted with this strange neoplasm.

The literature on immunotherapy of cancer is vast, but to the present day no panacea has been brought forth. Views and reviews have coursed from one extreme to another. Thus on the one hand, Strauss,¹⁷² writing in 1927, waved aside the immunotherapy of cancer as a matter of theoretical interest only and of no practical value while, during the past year, Murray²²⁹ has reported the seemingly successful palliation if not cure of a number of human cancers by the use of antiserum.

In 1903 Schmauch¹⁷⁹ had suggested the possibility that one day inoperable cases of choriocarcinoma might be treated successfully by the injection of specific antibodies. The proposal was echoed by Dickson²³⁰ in 1935 and, in the same year, Fortner and Owen²³¹ reported the start of experiments to treat choriocarcinoma in both male and female patients with serum from pregnant animals. In 1937, Frankl¹⁸⁰ proposed the use of normal human pregnancy serum, which he believed to contain syncytiolytic substances lacking in the blood of patients with choriocarcinoma. Indeed, two years later Richter²³² reported the regression of disease in a 25-year-old primipara given massive transfusions of whole pregnancy blood; he remained guarded

in his interpretations, however, pointing out that, although the clinical diagnosis and hormone assays were consistent with choriocarcinoma, there was inadequate histological evidence. In 1941, Acosta-Sison and Espaniola²² reported the recovery of a patient with choriocarcinoma of the uterus, rectoserosa, and lungs; the case was treated by hysterectomy, irradiation of the pulmonary metastases, and intramuscular injections of pregnancy serum. Although the patient survived, the authors did not feel that the value of serum injection in therapy had been established.

Barnes,²³⁴ in 1954, gave daily transfusions of *post-partum* blood to a 36-year-old Para 6, Gravid 9 with pulmonary metastases visible in chest films. This patient was treated also by hysterectomy, deep X-ray therapy, and synthetic estrogens, but eventually succumbed in the sixth week after the start of treatment.

By far the most imaginative attack has been made by Doniach *et al.*²⁷² The patient, a 31-year-old Para I, Gravid II, was found to have choriocarcinoma in the uterus and right broad ligament. After removal of the uterus and adnexa, she was given a series of injections of leukocytes from her husband and grafted with a small piece of his skin. Some months later she returned with vaginal nodules that were treated with radium locally. Over a year after her last delivery, she showed pulmonary masses that regressed after heavy X-ray treatment to the lungs. A recurrence of pulmonary disease was treated successfully with methotrexate. At the same time that she was being given routine therapy, serologic tests showed that she had developed antibodies against her husband's tissues, and the graft from his skin was rejected in 12 days, while that from a control survived for 23 days. Doniach and his colleagues could not credit the immunization procedure with having cured the patient, but thought that it nevertheless helped.

Although this effort to construct a therapeutic technique out of the known genetic differences between choriocarcinoma and its female host cannot be considered altogether successful, it represents a major advance in the rationale of chorionic tumor management. The approach of Doniach *et al.* takes into account more than the time-honored view of neoplasms as a colony of cells gone amuck. Future investigations of the natural history of choriocarcinoma, invasive and benign moles, and indeed of the normal trophoblast as well, might derive great profit through adoption of the cosmopolitan outlook expressed so clearly in the words of Schopper and Pliess:¹⁴ "We may now define chorionepithelioma malignum as a biological interrelationship between physiological chorionic epithelium which, because of insufficient fetal and maternal restraints, proliferates without hindrance and the maternal organism with its power and tendency to destroy the trophoblast. Thus chorionepithelioma can be contemplated as a *biological concept*, and delivered from the narrow and constrained perspective of a purely histological approach. Whenever we speak of a chorionepithelioma we shall intend henceforth not merely a local growth of chorionic epithelium but rather the *biological complex* of which the tumor is only a regional manifestation."

SUMMARY

To attempt a synopsis of the mechanisms underlying the regression of trophoblast is a simple task, for during the sixty-five years since the work of Schmorl and Marchand we have journeyed only a few short steps toward the long-sought goal of understanding just what makes one chorionic tumor fade away and disappear and another proliferate relentlessly until it finally overpowers and destroys its host.

The spontaneous regression of trophoblast under both physiological and pathological conditions has been attributed to a congeries of vaguely defined agents and factors, most of which are merely conceptual exercises. Indeed, one may argue with some cause that the fortuitous degeneration of a choriocarcinoma does not differ fundamentally from that of any other tumor. Among processes that might limit the growth of chorionic tissue specifically, a genetically governed autosensitization appears most likely. The migration of placental cells to ectopic sites, long known to occur rather frequently even in normal gestation, or perhaps the diffusion of soluble antigenic materials into the maternal circulation, might provide a stimulus for sensitization. The placenta is known to contain substances capable of eliciting the formation of antibodies, the best known being polysaccharide in general structure, but their precise nature and distribution are not known. Accumulating evidence in favor of a cross antigenicity between placental and renal tissues may disclose a direct link with the etiology of clinical toxemia. These aspects of the problem, as well as the possible relevance of recent investigations of so-called histocompatibility reactions in tissue transplantation, have been discussed in a general way.

We have permitted ourselves the luxury of loosely reined speculation, for comprehension of trophoblastic function in health and disease is still somewhat primeval and in need of vigorous and creative exploration. Thus, few answers can be supplied for the numerous questions that remain, and a review can go little further than to suggest attractive lines of future inquiry. One may work and hope for a definitive synthesis of both present and future knowledge.

However, the necessity for caution and even dour skepticism in the interpretation of clinical and experimental data cannot be exaggerated. No hypothesis, however rational and consonant with the personal sentiments of the investigator, should be blessed with unreserved acceptance to the arbitrary exclusion of foreign and conceivably more fruitful routes of attack. Whenever so tempted, one would do well to recall the familiar tale by Andersen and reflect that, when all was said and done, the emperor really was not wearing any clothes.

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References

1. DUNGER, R. 1905. Chorionepitheliom und Blasenmole. Beitr. pathol. Anat. u. allgem. Pathol. **37**: 279-377.
2. HÖRMANN, K. 1905. Zur Frage der Bösartigkeit und der Spontanheilung von Chorionepitheliomen. Zentr. Gynäkol. **29**: 466.
3. PARK, W. W. & J. C. LEES. 1950. Choriocarcinoma. A general review, with an analysis of five hundred and sixteen cases. A. M. A. Arch. Pathol. **49**: 205-241.
4. BARDAWIL, W. A., A. T. HERTIG & J. T. VELARDO. 1957. Regression of trophoblastic I. Hydatidiform mole; a case of unusual features, possibly metastasis and regression; review of literature. Obstet. & Gynecol. **10**: 614-625.
5. GREIN, E. 1904. Ein Fall von Chorionepithelioma malignum nebst einigen Bemerkungen über Spontanheilung und Therapie bei dieser Erkrankung. Arch. Gynaekol. **72**: 470-496.
6. CLEMMER, J. J. & G. H. HANSMANN. 1935. The origin of chorionepitheliomas and of emboli from trophoblastic fragments enclosed in the myometrium. Am. J. Obstet. Gynecol. **29**: 526-534.
7. PRYM, P. 1927. Spontanheilung eines bösartigen, wahrscheinlich chorionepitheliomatösen Gewächses im Hoden. Arch. pathol. Anat. u. Physiol. Virchow's **265**: 239-258.
8. WOODGER, J. H. 1952. Biology and Language. Tarner Lectures. 1949-50. Cambridge Univ. Press. : 8; 175-177. Cambridge, England.
9. BRAMBELL, F. W. R., W. A. HEMMINGS & M. HENDERSON. 1951. Antibodies and Embryos. Athlone Press, London, England.
10. DODDS, G. S. 1922. The area of the chorionic villi in the full-term human placenta. Anat. Record. **24**: 287-294.
11. DEES-MATTINGLY, M. 1936. Absorptive area and volume of chorionic villi in circumvallate placentas. Am. J. Anat. **59**: 485-507.
12. CHRISTOFFERSEN, A. K. 1934. La superficie des villosités choriales du placenta à la fin de la grossesse (étude d'histologie quantitative). Compt. rend. soc. biol. **117**: 641-644.
13. AMOROSO, E. C. 1955. The comparative anatomy and histology of the placental barrier. In Trans. First Conf. on Gestation. : 119-224. C. A. Villee, Jr., Ed. Josiah Macy, Jr. Found. New York, N. Y.
14. WISLOCKI, G. B. & E. W. DEMPSEY. 1955. Electron microscopy of the human placenta. Anat. Record. **123**: 133-168.
15. EASTMAN, N. J. 1956. Obstetrics. : 163. 11th ed. Appleton-Century-Crofts. New York, N. Y.
16. SCHNEIDER, C. L. 1954. Obstetric shock. Some interdependent problems of coagulation. Obstet. & Gynecol. **4**: 273-294.
17. WIEDOW, W. 1888. Ueber den Zusammenhang zwischen Albuminurie und Placentarkrankung. Z. Geburtshilfe u. Gynäkol. **14**: 387-404.
18. KLINE, B. S. 1951. Microscopic observations of the development of the human placenta. Am. J. Obstet. Gynecol. **61**: 1065-1074.

19. WISLOCKI, G. B. & E. W. DEMPSEY. 1946. Histochemical age-changes in normal and pathological placental villi (hydatidiform mole, eclampsia). *Endocrinology*. **38**: 90-109.
20. PAINE, C. G. 1957. Observations on placental histology in normal and abnormal pregnancy. *J. Obstet. Gynaecol. Brit. Empire*. **64**: 668-672.
21. MCKAY, D. G., A. T. HERTIG, E. C. ADAMS, & M. V. RICHARDSON. 1958. Histochemical observations on the human placenta. *Obstet. & Gynecol.* **12**: 1-36.
22. CHESLEY, L. C. & N. M. ALTER. 1951. Studies on surviving human placental tissue. III. The toxicity of placental perfusates. *Am. J. Obstet. Gynecol.* **61**: 1218-1231.
23. FRIEDLÄNDER, C. 1870. Physiologisch-anatomische Untersuchungen über den Uterus. Simmel. Leipzig, Germany.
24. FRIEDLÄNDER, C. 1876. Ueber die Innenfläche des Uterus post partum. *Arch. Gynaekol.* **9**: 22-28.
25. SCHMORL, C. G. 1893. Pathologisch-anatomische Untersuchungen über Puerperal-Eklampsie. Vogel. Leipzig, Germany.
26. SCHMORL, C. G. 1902. Zur Lehre von der Eklampsie. *Arch. Gynaekol.* **65**: 504-529.
27. SCHMORL, C. G. 1905. Ueber das Schicksal embolisch verschleppter Plazentazellen. *Verhandl. deut. pathol. ges.* **8**: 39-46.
28. SCHMORL, C. G. 1905. Ueber das Schicksal embolisch verschleppter Plazentazellen. *Zentr. Gynäkol.* **29**: 129-137.
29. LUBARSCH, O. 1893. Zur Lehre von der Parenchymzellenembolie. *Fortschr. Med.* **11**: 845-860.
30. LEUSDEN, P. 1895. Beitrag zur pathologischen Anatomie der Puerperal eklampsie. *Virchow's Arch. pathol. Anat. u. Physiol.* **142**: 1-45.
31. KASSJANOW. 1896. Zur Frage über die Lungen-Embolie mit Placentar-Riesenzellen. Inaug.-Diss. St. Petersburg. Cited by Maximow⁷⁸ and Schmorl.²⁷
32. ASCHOFF, L. 1893. Ueber capilläre Embolie von riesenkernhaltigen Zellen. *Virchow's Arch. pathol. Anat. u. Physiol.* **134**: 11-25.
33. VEIT, J. 1901. Ueber Deportation von Chorionzotten (Verschleppung von Zotten in mütterliche Blutbahnen). *Z. Geburtshilfe u. Gynäkol.* **44**: 466-504.
34. HITSCHMANN, F. 1904. Tubargravidität bei gleichzeitig bestehender akuter Entzündung der Tube. *Z. Geburtshilfe u. Gynäkol.* **53**: 1-13.
35. HITSCHMANN, F. 1904. Die Deportation der Zotten und ihre Bedeutung. *Z. Geburtshilfe u. Gynäkol.* **53**: 14-35.
36. FELLNER, O. O. 1905. Ueber das Verhalten der Gefäße bei Eileiterschwangerschaft. Autothrombose. *Arch. Gynaekol.* **74**: 481-512.
37. VEIT, J. 1902. Ueber Albuminurie in der Schwangerschaft. *Berlin. klin. Wochschr.* **39**: 513-516; 540-543.
38. VEIT, J. 1904. Verschleppung von Zotten und ihre Folgen. *Zentr. Gynäkol.* **28**: 1-9.
39. VEIT, J. 1905. Weitere Beobachtungen zur Lehre von der Zottenverschleppung. *Zentr. Gynäkol.* **29**: 807-808.¹⁵²
40. VEIT, J. 1905. Discussion of Liepmann.
41. VEIT, J. 1905. Die Verschleppung der Chorionzotten (Zottendeportation). Ein Beitrag zur Geburtshilflichen Physiologie und Pathologie. Bergmann. Wiesbaden, Germany.
42. SCHOLTEN, R. & J. VEIT. 1902. Weitere Untersuchungen über Zottendeportation und ihre Folgen. *Zentr. Gynäkol.* **26**: 169-171.
43. SCHOLTEN, R. & J. VEIT. 1903. Syncytiolyse und Hämolyse. *Z. Geburtshilfe u. Gynäkol.* **49**: 210-232.
44. NEUMANN, J. 1896. Beitrag zur Lehre vom "malignen Deciduom." *Monatsh. Geburtshilfe u. Gynaekol.* **3**: 387-412.
45. WILLIAMSON, H. 1903. A case of syncytioma malignum occurring in a woman who died thirty-one years ago; with some observations on the mode of invasion of these tumors. *J. Obstet. Gynaecol. Brit. Empire*. **4**: 306-311.

46. POTEN, W. & W. VASSMER. 1900. Beginnendes Syncytium mit Metastasen, beobachtet bei Blasenmolenschwangerschaft. *Arch. Gynaekol.* **61**: 205-276.
47. PARK, W. W. 1958. Experimental trophoblastic embolism in the lungs. *J. Pathol. Bacteriol.* **75**: 257-265.
48. BARDAWIL, W. A., B. L. TOY & A. T. HERTIG. 1959. The regression of trophoblastic. *Obstet. & Gynecol.* In preparation.
49. MARCUSE, P. M. 1954. Pulmonary syncytial giant cell embolism. Report of maternal death. *Obstet. & Gynecol.* **3**: 210-213.
50. TROTTER, R. F. & H. L. TIECHE. 1956. Maternal death due to pulmonary embolism of trophoblastic cells. *Am. J. Obstet. Gynecol.* **71**: 1114-1118.
51. ARNOLD, H. A. & A. R. BAINBOROUGH. 1957. Subacute cor pulmonale following trophoblastic pulmonary embolism. *Can. Med. Assoc. J.* **76**: 478-482.
52. KOLLMANN, J. 1901. Kreislauf der Placenta, Chorionzotten, und Telogonion. *Z. Biol.* **42**: 1-30.
53. WISLOCKI, G. B. & E. W. DEMPSEY. 1948. The chemical histology of the human placenta and decidua with reference to mucopolysaccharides, glycogen, lipids, and acid phosphatase. *Am. J. Anat.* **83**: 1-43.
54. WISLOCKI, G. B. 1943. Some observations on the cytology of the trophoblast. *Proc. Conf. Problems of Human Fertility*: 134-143. Banta Publ. Co. Menasha, Wis.
55. BOYD, J. D. & A. F. W. HUGHES. 1954. Observations on human chorionic villi using the electron microscope. *J. Anat.* **88**: 356-362.
56. ULESKO-STROGANOWA, K. 1896. Beiträge zur Lehre vom mikroskopischen Bau der Placenta. *Monatschr. Geburtshilfe u. Gynaekol.* **3**: 222-240.
57. VIRCHOW, R. 1856. Ueber die Bildung der Placenta. *Ges. Abhandl.* **II**: 788. *Cited in Veit.*^{33, 41}
58. POTEN, W. 1902. Die Verschleppung der Chorionzotten. *Arch. Gynaekol.* **66**: 590-617.
59. MEYER-RÜEGG, H. 1903. Ein Fall von Placentaradhärenz. *Z. Geburtshilfe Gynaekol.* **50**: 141-152.
60. BAUEREISEN, A. 1904. Beitrag zur Frage der Placentaradhärenz. *Z. Geburtshilfe u. Gynaekol.* **53**: 310-322.
61. FAÇEE SCHEFFER. 1902. Inaug. Diss. Leiden. *Cited in Veit.*³³
62. BOYD, J. D. 1956. Morphology and physiology of the uteroplacental circulation. *In Trans. 2nd Conf. on Gestation*: 132-194. C. A. Villee, Jr., Ed. Josiah Macy Jr. Found. New York, N. Y.
63. RAMSEY, E. M. 1956. Distribution of arteries and veins in the mammalian placenta. *Ibid.*: 229-251.
64. NOVAK, E. & E. R. NOVAK. 1958. *Gynecologic and Obstetric Pathology*. : 51. 4th ed. Saunders. Philadelphia, Pa.
65. KISTNER, R. W., W. R. GRAF & R. E. JOHNSTONE. 1950. Pulmonary embolism with particulate matter of the amniotic fluid. A report of two cases with a review of the literature. *Obstet. Gynecol. Survey.* **5**: 629-647.
66. ENGLE, E. T. 1956. Discussion of Amoroso.¹³
67. WISLOCKI, G. B. 1955. Discussion of L. B. Flexner. *Trans. 1st Conf. on Gestation*: 48. Josiah Macy, Jr. Found. New York, N. Y.
68. WISLOCKI, G. B. 1956. Discussion of Ramsey.⁶³
69. SIEGMUND, H. 1926. Über das Schicksal eingeschwemmter Reticulo-Endothelien (Bluthistiocyten) in den Lungengefäßen. Ein weiterer Beitrag zur Entstehung von Gefäßwandgranulomen. *Z. Exptl. Med.* **50**: 73-84.
70. JEFFCOATE, T. N. A. 1955. The vertebral venous drainage of the pelvis. *J. Obstet. Gynaecol. Brit. Empire* **62**: 244-246.
71. SPANNER, R. 1935. Mütterlicher und kindlicher Kreislauf der menschlicher Placenta und seine Strohmbahnen. *Z. Anat. Entwicklungsgeschichte.* **105**: 163-243.
72. FALKNER, N. 1939. Circulation of the maternal blood through the placenta. *Irish J. Med. Sci.* **6**: 59-65.
73. EARN, A. A. & D. NICHOLSON. 1952. The placental circulation, maternal and fetal. *Am. J. Obstet. Gynecol.* **63**: 1-5.

74. MILLER, J. 1923-1924. A case of malignant hydatidiform mole with pulmonary metastases. *Edinburgh Med. J. Suppl.* : 217-224.
75. COE, J. I. & S. REED. 1958. An invasive hydatidiform mole metastatic to the lungs. *Obstet. & Gynec.* In press.
76. HARRISON, C. V. 1958. The pathology of the pulmonary vessels in pulmonary hypertension. *Brit. J. Radiol.* **31**: 217-226.
77. OBER, W. B. 1958. In preparation.
78. MAXIMOW, A. 1898. Zur Lehre von der Parenchymzellen-Embolie der Lungenarterie. *Virchow's Arch. pathol. Anat. u. Physiol.* **151**: 297-318.
79. SCHNEIDER, C. L. 1950. Complications of late pregnancy in rabbits induced by experimental placental trauma. *Surg. Gynecol. Obstet.* **90**: 613-622.
80. LUBARSCH, O. 1898. Ueber Knochenmarkgewebs Embolie. *Virchow's Arch. pathol. Anat. u. Physiol.* **151**: 546-549.
81. FRIEDHEIM, E. A. H. 1929. Die Züchtung von menschlichem Chorionepithel *in vitro*. Ein Beitrag zur Lehre vom Chorionepitheliom. *Virchow's Arch. pathol. Anat. u. Physiol.* **272**: 217-244.
82. GEY, G. O., G. E. SEEGAR JONES & L. M. HELLMAN. 1938. The production of gonadotrophic substance (prolan) by placental cells in tissue culture. *Science.* **88**: 306-307.
83. STEWART, H. L., JR., M. E. SANO & T. L. MONTGOMERY. 1948. Hormone secretion by human placenta grown in tissue culture. *J. Clin. Endocrinol.* **8**: 175-188.
84. WALTZ, H. K., W. W. TULLNER, V. J. EVANS, R. HERTZ & W. R. EARLE. 1953. Chorionic gonadotropin recovery from hydatid mole grown in tissue culture. *Abstr. Proc. Am. Assoc. Cancer Research.* **1**: 57.
85. UYENO, H. 1933. Über die Gewebezüchtung von einem Chorionepithelioma malignum. *Nippon Fujinkagakkai Zassi.* **28**: 58-59.
86. KIDO, I. 1936. Über heteroplastische Transplantationsversuche mit menschlicher Placenta auf Tiere. I. Mitteilung: Über einen Fall von gelungener Transplantation der menschlichen Placenta in die Augenvorderkammer eines Kaninchens. *Zentr. Gynäkol.* **60**: 1162-1166.
87. KIDO, I. 1937. Die menschliche Placenta als Produktionsstätte des sogenannten Hypophysenvorderlappenhormon. *Zentr. Gynäkol.* **61**: 1551-1555.
88. BROWNING, H. C. 1949. Homologous and heterologous growth of transplants of various tissues during the course of development of the mouse. *Cancer.* **2**: 646-672.
89. GROBSTEIN, C. 1955. Discussion of L. B. Flexner. The functional role of the placenta. *Trans. First Conf. on Gestation.* : 51. Claude A. Villee, Ed. Josiah Macy, Jr. Found. New York, N. Y.
90. LITT, S. 1933. Autotransplantation of placenta to the anterior chamber of the eye and its effect on lactation. *Am. J. Obstet. Gynecol.* **26**: 37-45.
91. FAWCETT, D. W., G. B. WISLOCKI & C. M. WALDO. 1947. The development of mouse ova in the anterior chamber of the eye and in the abdominal cavity. *Am. J. Anat.* **81**: 413-444.
92. FAWCETT, D. W. 1950. The development of mouse ova under the capsule of the kidney. *Anat. Record.* **108**: 71-91.
93. TOOLAN, H. W. 1953. Growth of human tumors in cortisone-treated laboratory animals: The possibility of obtaining permanently transplantable human tumors. *Cancer Research.* **13**: 389-394.
94. TOOLAN, H. W. 1955. The possible role of cortisone in overcoming resistance to the growth of human tissues in heterologous hosts. *Ann. N. Y. Acad. Sci.* **59**(3): 394-399.
95. HANDLER, A. H. & G. YERGANIAN. 1954. The transplantation of human teratomas and embryonal tissues in cortisone-treated animals. *Proc. Am. Assoc. Cancer Research. Abstr.* **1**: 18.
96. TEACHER, J. H. 1927. On involution of the uterus postpartum. *J. Obstet. Gynaecol. Brit. Empire.* **34**: 1-27.
97. RUTHERFORD, R. N. & A. T. HERTIG. 1945. Non-involution of the placental site. *Am. J. Obstet. Gynecol.* **49**: 378-384.

98. WARE, H. H., JR. 1936. Late extra-uterine pregnancy. N. Y. State J. Med. **39**: 1943-1950.
99. REED, C. B. 1950. Abdominal pregnancy. Full term with surviving infant and mother. Texas J. Med. **46**: 379-381.
100. MILLEN, R. S. 1956. The fate of the placenta left *in situ* following the delivery of a fetus in abdominal pregnancy. Am. J. Obstet. Gynecol. **71**: 1348-1350.
101. LAZARUS, J. A. & A. SCHIFRIN. 1942. An unusual case of benign multiple chorionic villi implants in peritoneal cavity accompanied by hemoperitoneum. Ann. Surg. **115**: 93-101.
102. NEWTON, W. H. 1935. "Pseudo-parturition" in the mouse, and the relation of the placenta to post-partum oestrus. J. Physiol. **84**: 196-207.
103. VAN WAGENEN, G. & W. H. NEWTON. 1943. Pregnancy in the monkey after removal of the fetus. Surg. Gynecol. Obstet. **77**: 539-543.
104. COPPLESON, M. 1958. Hydatidiform mole and its complications. J. Obstet. Gynaecol. Brit. Empire. **65**: 238-252.
105. RISEL, W. 1903. Über das maligne Chorionepitheliom und die analogen Wucherungen in Hodenteratomen. : 56-57. S. Hirzel. Leipzig, Germany.
106. TEACHER, J. H. 1908. On the development and natural healing of secondary tumours of chorionepithelioma malignum. J. Pathol. Bacteriol. **12**: 487-506.
107. BREWS, A. 1939. Hydatidiform mole and chorion-epithelioma. J. Obstet. Gynaecol. Brit. Empire. **46**: 813-835.
108. BREWS, A. 1950. Some aspects of chorionepithelioma. J. Obstet. Gynaecol. Brit. Empire. **57**: 317-321.
109. FEINER, D. 1935. Chorionepithelioma with a long latent period. Am. J. Obstet. Gynecol. **29**: 840-844.
110. WHITE, T. G. E. 1955. A chorion-epithelioma of the uterus in a post-menopausal woman. J. Obstet. Gynaecol. Brit. Empire. **62**: 372-374.
111. CHOWN, B. 1954. Anaemia from bleeding of the foetus into the mother's circulation. Lancet. **1**: 1213-1215.
112. DUNSFORD, I. 1957. Proof of foetal antigens entering the maternal circulation. Vox Sanguinis. **2**: 125-127.
113. HEDENSTEDT, S. & J. NAESLUND. 1946. Investigations of the permeability of the placenta with the help of elliptocytes. Acta Med. Scand. Suppl. **170**: 126-134.
114. NAESLUND, J. & G. NYLIN. 1946. Investigations on the permeability of the placenta with the aid of red blood corpuscles tagged with radio-active phosphorus. Acta Med. Scand. Suppl. **170**: 390-398.
115. MENGERT, W. F., C. S. RIGHTS, C. R. BATES, JR., A. F. REID, G. R. WOLF & G. NABORS. 1955. Placental transmission of erythrocytes. Am. J. Obstet. Gynecol. **69**: 678-685.
116. WARD, W. H. & W. W. GUNTHER. 1956. Lupus erythematosus. Australasia J. Dermatol. **3**: 159-170.
117. BARDAWIL, W. A., B. L. TOY, N. GALINS & T. B. BAYLES. 1958. Disseminated lupus erythematosus, scleroderma, and dermatomyositis as manifestations of sensitization to DNA-protein. I. An immunohistochemical approach. Am. Pathol. **34**: 607-629.
118. GUTTMACHER, A. F. 1943. A case of severe scleroderma with successful delivery. Urol. and Cutaneous Rev. **47**: 107-108.
119. CRAWFORD, G. M. & R. W. LEEPER. 1950. Diseases of the skin in pregnancy. A.M.A. Arch. Dermatol. Syphilol. **61**: 753-771.
120. KASDON, S. C. 1953. Abdominal pruritis in pregnancy. Am. J. Obstet. Gynecol. **65**: 320-324.
121. MONTGOMERY, T. S. 1931. The etiology and significance of necrosis (infarction) of the placenta; a biologic and histologic study. Am. J. Obstet. Gynecol. **23**: 157-174.
122. TENNEY, B., JR. 1935. A study of the collagen of the placenta. Am. J. Obstet. Gynecol. **29**: 819-825.
123. TENNEY, B., JR. 1936. Syncytial degeneration in normal and pathologic placenta. Am. J. Obstet. Gynecol. **31**: 1024-1028.

124. TENNEY, B., JR. & F. PARKER, JR. 1940. The placenta in toxemia of pregnancy. *Am. J. Obstet. Gynecol.* **39**: 1000-1005.
125. GRAY, J. D., C. TUPPER & J. A. ROWSE. 1958. The problem of spontaneous abortion. V. The genesis of spontaneous abortion. *Am. J. Obstet. Gynecol.* **75**: 43-52.
126. AMOROSO, E. C. 1952. Placentation. Chap. 15. *In*: Marshall; Physiology of Reproduction. **2**: 127-311. A. S. Parkes, Ed. Longmans, Green and Co. London, England.
127. MARCUS, M. B., L. J. CIBLEY, M. L. BRANDT, L. MILLMAN & D. BARLAS. 1958. Circulating eosinophils in labor and puerperium. *Am. J. Obstet. Gynecol.* **75**: 11-15.
128. SPEIRS, R. S. 1958. Advances in the knowledge of the eosinophil in relation to antibody formation. *Ann. N. Y. Acad. Sci.* **73**(1): 283-306.
129. NOVELL, H. A. & Q. C. DE HAAN. 1958. Toxemia of pregnancy. Treatment with hypotensive drugs and relationship to anaphylaxis. *Obstet. Gynecol. Survey.* **12**: 154-163.
130. BAĐAWY, A. H. & B. GABRAWY. 1958. Antihistaminics in the management of abortion. *J. Obstet. Gynaecol. Brit. Empire.* **65**: 260-265.
131. FEHLING, H. 1886. Ueber habituelles Absterben der Frucht bei Nierenerkrankung der Mutter. *Arch. Gynaekol.* **27**: 300-306.
132. DOBROWOLSKI, M. S. 1903. O cytotoksynie lozyskowej. *Bull. intern. acad. sci. Cracovie.* No. **5**: 256-260.
133. ASCOLI, A. 1902. Zur experimentellen Pathogenese der Eklampsie. *Zentr. Gynäkol.* **26**: 1321-1326.
134. WORMSER, E. 1904. Zur modernen Lehre von der Eklampsie. *Münch. med. Wochschr.* **51**: 7-10.
135. WEICHARDT, W. 1902. Experimentelle Studien über die Eklampsie. *Deut. med. Wochnschr.* **28**: 624-626.
136. WORMSER, E. & A. LABHARDT. 1904. Weitere Untersuchungen zur modernen Lehre der Eklampsie. *Münch. med. Wochschr.* **51**: 2285-2287.
137. COHEN, H. R. & A. J. NEDZEL. 1940. Specific action of an antiserum for placental proteins on placenta and normal progress of pregnancy. *Proc. Soc. Exptl. Biol. Med.* **43**: 249-250.
138. SEEGAL, B. C. & E. N. LOEB. 1940. Effect of anti-placenta serum on development of foetus in the pregnant rat. *Proc. Soc. Exptl. Biol. Med.* **45**: 248-252.
139. SEEGAL, B. C., M. W. HASSON, E. N. LOEB & A. I. KNOWLTON. 1951. Production of cytotoxic nephritis in the dog by specific antiplacenta serum. *Federation Proc.* **10**: 418.
140. SEEGAL, B. C., M. W. HASSON, E. C. GAYNOR & M. S. ROTHENBERG. 1955. Glomerulonephritis produced in dogs by specific antisera. I. The course of the disease resulting from the injection of rabbit antidog-placenta serum or rabbit antidog-kidney serum. *J. Exptl. Med.* **102**: 789-805.
141. BEVANS, M., B. C. SEEGAL & R. KAPLAN. 1955. Glomerulonephritis produced in dogs by specific antisera. II. Pathologic sequences following the injection of rabbit antidog-placenta serum or rabbit antidog-kidney serum. *J. Exptl. Med.* **102**: 807-821.
142. SEEGAL, B. C. & E. N. LOEB. 1943. The production of chronic progressive nephritis in the rat following the initial injection of specific anti-placenta serum. I. Clinical findings. *Federation Proc.* **2**: 101-102.
143. LOEB, E. N. & B. C. SEEGAL. 1943. The production of chronic nephritis in the rat following the initial injection of anti-placenta serum. II. Pathological findings. *Federation Proc.* **2**: 99-100.
144. SEEGAL, B. C. & E. N. LOEB. 1946. The production of chronic glomerulonephritis in rats by the injection of rabbit anti-rat-placenta serum. *J. Exptl. Med.* **84**: 211-222.
145. MCCAUGHEY, W. T. E. 1955. The nephrotoxic action of anti-placenta serum in rats. *J. Obstet. Gynaecol. Brit. Empire.* **62**: 863-869.
146. BAXTER, J. H. & H. C. GOODMAN. 1956. Nephrotoxic serum nephritis in rats.

- I. Distribution and specificity of the antigen responsible for the production of nephrotoxic antibodies. *J. Exptl. Med.* **104**: 467-485.
147. LIN, H. A. C. 1947. Is toxemia of pregnancy an allergic reaction? *Am. J. Obstet. Gynecol.* **54**: 97-101.
148. KAWASOYE. 1904. Ueber die biochemische Diagnose der Schwangerschaft. Inaug. Diss. Erlangen. Cited in Veit⁴¹ and in Wormser and Labhardt.¹³⁶
149. ZIRONI, G. 1905. Experimenteller Beitrag zur Pathogenese der Eklampsie (Abstract). *Zentr. Gynäkol.* **29**: 188.
150. ARONSON. 1905. Discussion of W. Liepmann. Neuere biologische Erfahrungen über das Wesen der Eklampsie. *Z. Geburtshilfe u. Gynäkol.* **56**: 243-247.
151. WEICHARDT, W. 1903. Ueber die Aetiologie der Eklampsie. *Z. Geburtshilfe u. Gynäkol.* **50**: 25-29.
152. LIEPMANN, W. 1903. Ueber ein für menschliche Placenta spezifisches Serum. *Deut. med. Wochschr.* **29**: 80-81.
153. BASSETT, C. A. L., D. H. CAMPBELL, V. J. EVANS & W. R. EARLE. 1957. The cytotoxic activity of rabbit immune globulin prepared from tissue cultures and whole human placenta. *J. Immunol.* **78**: 79-93.
154. GREENSPON, S. A. & C. A. KRAKOWER. 1950. Direct evidence for the antigenicity of the glomeruli in the production of nephrotoxic serums. *A. M. A. Arch. Pathol.* **49**: 291-297.
155. CRUICKSHANK, B. & A. G. S. HILL. 1953. The histochemical identification of connective-tissue antigen in the rat. *J. Pathol. Bacteriol.* **66**: 283-289.
156. MELLORS, R. C., J. ARIAS-STELLA, M. SIEGEL & D. PRESSMAN. 1955. Analytical pathology. II. Histopathological demonstration of glomerular-localizing antibodies in experimental glomerulonephritis. *Am. J. Pathol.* **31**: 687-716.
157. GOODMAN, M., S. A. GREENSPON & C. A. KRAKOWER. 1955. The antigenic composition of the various anatomic structures of the canine kidney. *J. Immunol.* **75**: 96-104.
158. KRAKOWER, C. A. & S. A. GREENSPON. 1958. The localization of the "nephrotoxic" antigen(s) in extraglomerular tissues. Observations including a measure of concentration in certain locales. *A. M. A. Arch. Pathol.* **66**: 364-383.
159. PRESSMAN, D. & L. KORNGOLD. 1957. Localizing properties of anti-placenta serum. *J. Immunol.* **78**: 75-78.
160. TOY, B. L. 1957. The regression of trophoblast. An immuno-histochemical approach. Thesis. Harvard School of Dental Medicine. Boston, Mass.
161. BARDAWIL, W. A., B. L. TOY & A. T. HERTIG. 1958. Localization of homologous plasma proteins in the human placenta by fluorescent antibody. *Am. J. Obstet. Gynecol.* **75**: 708-717.
162. THORNS, R. D., J. A. MACLAREN, C. C. ROBY & D. E. REID. 1958. Unpublished findings. Personal communication from C. C. Roby.
163. SMITH, G. V., O. W. SMITH & S. L. ROMNEY. 1952. Trials with penicillin in the treatment of pre-eclampsia and eclampsia. *Am. J. Obstet. Gynecol.* **63**: 1185-1191.
164. LAPAN, B. & I. A. SHEY. 1954. Placental globulin skin sensitivity, with special reference to toxemias of pregnancy. *Am. J. Obstet. Gynecol.* **67**: 528-541.
165. SCANU, A., L. A. LEWIS & I. H. PAGE. 1958. Studies on the antigenicity of β - and α -lipoproteins of human serum. *J. Exptl. Med.* **108**: 185-196.
166. KAKU, M. 1953. Placental polysaccharide and the aetiology of the toxemia of pregnancy. *J. Obstet. Gynaecol. Brit. Empire.* **60**: 148-156.
167. MILAZZO, S. C. 1957. A study of the immunological properties of reticulin. *Pathol. Bacteriol.* **73**: 527-537.
168. WILLIS, R. A. 1952. The Spread of Tumours in the Human Body. : 85. Butterworth. London, England.
169. GAYLORD, H. R. & G. H. A. CLOWES. 1906. On spontaneous cure of cancer. *Surg. Gynecol. Obstet.* **2**: 633-658.
170. FRAUCHIGER, R. 1929. Zur Frage der Spontanheilung von Carcinomen. Kritische Zusammenstellung. *Z. Krebsforsch.* **29**: 516-548.
171. ROHDENBURG, G. L. 1918. Fluctuations in the growth energy of malignant tumors.

- in man, with especial reference to spontaneous recession. *J. Cancer Research* **3**: 193-225.
172. STRAUSS, O. 1927. Über die Spontanheilung des Carcinoms. *Z. Krebsforsch.* **24**: 367-385.
173. EVERSON, T. C. & W. H. COLE. 1956. Spontaneous regression of cancer: preliminary report. *Ann. Surg.* **144**: 366-383.
174. HANOVER. 1852. *Das Epithelium.* Cited in.¹⁷¹
175. ZALKA, E. DE. 1928. Concerning ectopic chorionepithelioma. Report of two cases. *Am. J. Pathol.* **4**: 59-74.
176. PETERS, H. 1902. Zur Lehre vom primären Chorionepithelium der Scheide nebst einem Falle von Recidiv nach Exstirpation des Scheidenknotens. *Zentr. Gynäkol.* **26**: 769-777.
177. VON VELITS, D. 1905. Weitere Studien über die Spontanheilung des Chorionepithelioms. *Z. Geburtshilfe u. Gynäkol.* **56**: 378-396.
178. ANSPACH, B. M. & J. HOFFMAN. 1931. Perforating chorionepithelioma of the uterus with free intraperitoneal hemorrhage. *Am. J. Obstet. Gynecol.* **22**: 239-246.
179. SCHMAUCH, G. 1903. Das Syncytioma malignum vaginale p.p. mature ohne Geschwulstbildung im Uterus und seine Aetiologie. *Z. Geburtshilfe u. Gynäkol.* **49**: 387-416.
180. FRANKL, O. 1937. Chorionepitheliom: ein kritischer Bericht. *Ber. Ges. Gynäkol. u. Geburtshilfe.* **33**: 385-401.
181. VON MICKULICZ-RADECKI, F. 1928. Bericht über 2 klinisch beobachtete Fälle von Chorionepitheliom. *Z. Geburtshilfe u. Gynäkol.* **92**: 216-224.
182. WITEBSKY, E. & N. R. ROSE. 1956. Studies on organ specificity. IV. Production of rabbit thyroid antibodies in the rabbit. *J. Immunol.* **76**: 408-416.
183. WITEBSKY, E., N. R. ROSE, K. TERPLAN, J. R. PAINE & R. W. EGAN. 1957. Chronic thyroiditis and autoimmunization. *J. Am. Med. Assoc.* **164**: 1439-1447.
184. ROITT, M., D. DONIACH, P. N. CAMPBELL & R. V. HUDSON. 1956. Auto-antibodies in Hashimoto's disease (lymphadenoid goitre). *Lancet.* **2**: 820-821.
185. FREUND, J., M. M. LIPTON & G. E. THOMPSON. 1953. Aspermatogenesis in the guinea pig induced by testicular tissue and adjuvants. *J. Exptl. Med.* **97**: 711-726.
186. VOISIN, G. A., F. TOULLET & P. MAURER. 1958. The nature of tissular antigens, with particular reference to autosensitization and transplantation immunity. *Ann. N. Y. Acad. Sci.* **73**(3): 726-744.
187. WILSON, L. 1954. Sperm agglutinins in human semen and blood. *Proc. Soc. Exptl. Biol. Med.* **85**: 652-655.
188. BLAKELY, S. B. 1937. The diagnosis of the sex of the human fetus *in utero*. *Am. J. Obstet. Gynecol.* **34**: 322-335.
189. RUYCK, R. DE. 1950. De la présence d'éléments corpusculaires particuliers et filtrables dans une mole hydatiforme compliquée de chorioépithéliome. *Compt. rend.* **231**: 1106-1107.
190. TEACHER, J. H. 1903. On chorionepithelioma and the occurrence of chorionepitheliomatous and hydatidiform mole-like structures in teratomata. A pathological and clinical study. *J. Obstet. Gynaecol. Brit. Empire.* **4**: 1-64.
191. LIEPMANN, W. 1905. Zur Biologie der Placenta. *Z. Geburtshilfe u. Gynäkol.* **56**: 232-243.
192. SCHOPPER, W. & G. PLIESS. 1949. Über Chorionepitheliosis. Ein Beitrag zur Genese, Diagnostik, und Bewertung ektopischer chorionepithelialer Wucherungen. *Virchow's Arch. pathol. Anat. u. Physiol.* **317**: 347-384.
193. SCHUSTER, A. 1952. Genese und Therapie des Chorionepithelioma malignum. *Arch. Gynaekol.* **181**: 477-499.
194. SCHOPPER, W. 1952. Das Chorionepitheliom: eine biologische Betrachtung. *Deut. med. Wochschr.* **77**: 1218-1221.
195. DOWLING, E. A. 1957. Choriocarcinoma and hydatidiform mole as tissue transplants. *Southern Med. J.* **50**: 211-212.
196. GRENE, H. S. N. 1952. The significance of the heterologous transplantability of human cancer. *Cancer.* **5**: 24-44.

197. SMALBRAAK, J. 1957. Trophoblastic Growths. A Clinical, Hormonal and Histopathologic Study of Hydatidiform Mole and Chorionepithelioma. Elsevier Amsterdam, Holland.
198. PEARSON, O. H., B. S. RAY, C. C. HARROLD, C. D. WEST, M. C. LI, J. P. MACLEAN & M. B. LIPSETT. 1956. Hypophysectomy in treatment of advanced cancer. *Am. Med. Assoc.* **161**: 17-21.
199. WEISS, P. 1952. Self-regulation of organ growth by its own products. *Science* **115**: 487-488.
200. ROSE, S. M. 1958. Failure of self-inhibition in tumors. *J. Natl. Cancer Inst.* **20**: 653-664.
201. HELFF, O. M. 1955. Mammary tumor regression by extracts of histolyzed amphibian skin and gills. *Proc. Soc. Exptl. Biol. Med.* **88**: 315-317.
202. NACE, G. W. 1955. Development in the presence of antibodies. *Ann. N. Y. Acad. Sci.* **60**(7): 1038-1055.
203. STURTEVANT, A. H. 1944. Can specific mutations be induced by serologic methods? *Proc. Natl. Acad. Sci.* **30**: 176-178.
204. EMERSON, S. 1944. The induction of mutations by antibodies. *Proc. Natl. Acad. Sci.* **30**: 179-183.
205. MALMGREN, R. A. 1958. Influence of antigenic factors in the production of antitumor cytotoxic sera. *J. Natl. Cancer Inst.* **20**: 417-429.
206. SNELL, G. D. 1948. Methods for the study of histocompatibility genes. *J. Genet.* **49**: 87-103.
207. SNELL, G. D. 1952. The immunogenetics of tumor transplantation. *Cancer Research* **12**: 543-546.
208. GORER, P. A. 1937. The genetic and antigenic basis of tumor transplantation. *J. Pathol. Bacteriol.* **44**: 691-697.
209. HAUSCHKA, T. S. 1953. Methods of conditioning the graft in tumor transplantation. *J. Natl. Cancer Inst.* **14**: 723-739.
210. HAUSCHKA, T. S. & A. LEVAN. 1953. Inverse relationship between chromosome ploidy and host specificity of sixteen transplantable tumors. *Exptl. Cell Research* **4**: 457-467.
211. AMOS, D. B. 1955. Serological differences between comparable diploid and tetraploid lines of three mouse ascites tumors. *Ann. N. Y. Acad. Sci.* **63**(5): 706-710.
212. LEVAN, A. 1956. Chromosomes in cancer tissue. *Ann. N. Y. Acad. Sci.* **63**(5): 774-792.
213. GORER, P. A. 1948. The significance of studies with transplanted tumours. *Brit. J. Cancer.* **2**: 103-107.
214. HAUSCHKA, T. S. & J. SCHULTZ. 1954. Cytologic aspects of immunogenetic specificity. *Transplantation Bull.* **1**: 203-206.
215. FELDMAN, M. & L. SACHS. 1958. Immunogenetic properties of tumors that have acquired homotransplantability. *J. Natl. Cancer Inst.* **20**: 513-539.
216. KAY, H. E. M. 1957. A and B antigens of normal and malignant cells. *Brit. J. Cancer.* **11**: 409-414.
217. EICHWALD, E. J. & C. R. SILMSER. 1955. Discussion of skin-graft data. *Transplantation Bull.* **2**: 148-149.
218. EICHWALD, E. J., C. R. SILMSER & I. WEISSMAN. 1958. Sex-linked rejection of normal and neoplastic tissue. I. Distribution and specificity. *J. Natl. Cancer Inst.* **20**: 563-575.
219. HAUSCHKA, T. S. 1955. Probable Y-linkage of a histocompatibility gene. *Transplantation Bull.* **2**: 154-155.
220. PEER, L. A. 1958. Behavior of skin grafts exchanged between parents and offspring. *Ann. N. Y. Acad. Sci.* **73**(3): 584-589.
221. SCHULTZ, J. 1956. The relation of the heterochromatic chromosome regions to the nucleic acids of the cell. *Symposia Quant. Biol.* **21**: 307-328.
222. LEVIJ, I. S. 1955. Enkele toepassingen van de geslachtsbepaling aan celkernen. *Ned. Tijdschr. Geneesk.* **99**: 1477-1482.
223. OOSTERHUIS, W. W. & I. S. LEVIJ. 1957. Geslachtschromatine in chorion-epitheliomen en in carcinomen van de huid. *Ned. Tijdschr. Geneesk.* **101**: 901-903.

224. BILLINGHAM, R. E., L. BRENT & P. B. MEDAWAR. 1956. Quantitative studies on tissue transplantation immunity. III. Actively acquired tolerance. Phil. Trans. Roy. Soc. London. **B239**: 357-414.
225. ZEIDMAN, I., M. McCUTCHEON & D. R. COMAN. 1950. Factors affecting the number of tumor metastases. Experiments with a transplantable mouse tumor. Cancer Research. **10**: 357-359.
226. FLEXNER, S. & J. W. JOBLING. 1907. On the promoting influence of heated tumor emulsions on tumor growth. Proc. Soc. Exptl. Biol. Med. **4**: 156-157.
227. KALISS, N. & N. MOLOMUT. 1952. The effect of prior injections of tissue anti-serums on the survival of cancer homoigrafts in mice. Cancer Research. **12**: 110-112.
228. KALISS, N. & B. F. BRYANT. 1958. Factors determining homograft destruction and immunological enhancement in mice receiving successive tumor inocula. J. Natl. Cancer Inst. **20**: 691-704.
229. MURRAY, G. 1958. Experiments in immunity in cancer. Can. Med. Assoc. J. **79**: 249-259.
230. DICKSON, J. G. 1935. Chorio-epithelioma: should serum from the female in the puerperium and pregnancy be given a therapeutic trial? U. S. Naval Med. Bull. **33**: 358-362.
231. FORTNER, H. C. & S. E. OWEN. 1935. Chorionepithelioma in the male. Am. J. Cancer. **25**: 89-97.
232. RICHTER, W. E. 1939. Transfusion von Schwangerenblut bei Chorionepitheliom. Zentr. Gynäkol. **63**: 1567-1573.
233. ACOSTA-SISON, H. & N. ESPANIOLA. 1941. Clinicopathological study from thirty-two cases of chorionepithelioma. Am. J. Obstet. Gynecol. **42**: 878-884.
234. BARNES, L. W. 1954. Choriocarcinoma treated with postpartum blood. Obstet. & Gynecol. **4**: 227-230.
235. DONIACH, I., J. H. CROOKSTON & T. I. COPE. 1958. Attempted treatment of a patient with chorioncarcinoma by immunization with her husband's cells. J. Obstet. Gynaecol. Brit. Empire. **65**: 553-556.

Discussion of the Paper

W. W. PARK: W. A. Bardawil and B. L. Toy are to be congratulated on finding apparently normal chorionic villi within the lungs of 2 patients in their series; I think it very possible that these are the first villi ever to be seen within the lung in nonmolar pregnancy. The few examples that I have seen have been in patients who had had a hydatidiform mole, and the same thing applies, I think, to such examples of intrapulmonary villi as have been described in the literature. The series of pregnancy lungs examined by Bardawil and Toy numbered 109; a series I examined numbered 120; and I have knowledge of a further series of approximately 50 (unpublished) examined by a colleague, Harold Attwood, of Dundee, Scotland. These 3 series comprise almost 300 cases, and only in 2 of them have chorionic villi been detected. I agree entirely with Bardawil and Toy that statements made in current texts about the frequent or even invariable passage of villi to the lungs during normal pregnancy stand in need of correction in future editions.

In respect to the frequency of occurrence of trophoblastic embolism of the lungs, the Boston, Mass., figures represent an incidence of 52 per cent. In my series of 120 cases, trophoblast was present in 53 cases, or 44 per cent; therefore, a round figure of approximately 50 per cent possibly is a true measure of the frequency. On the other hand, it seemed quite clear from

the examination of my material that, the greater the number of blocks and sections of material examined, the greater was the likelihood of finding trophoblast, and I suspect that, if there had been available, for example, 10 nonserial sections from each of 10 blocks in every patient, the total incidence would have been closer to 70 per cent.

However, apart from the frequency of occurrence of trophoblast within the lungs, there is appreciable variation in the amount of trophoblast that is seen in sections from the positive cases, and I should like to ask Bardach and Toy whether they made any attempt to measure this. Since the variation in amount in the 53 cases existed in my series, it seemed desirable to try to measure the amount of trophoblast in each case, with a view to determining whether variations in quantity were associated in any way with different clinical states. The amount of trophoblast was assessed by using a trophoblastic index, that is, by counting the number of particles of trophoblast seen in 1000 sq. mm. of sectioned lung.

There are 4 factors that might be expected, *a priori*, to have some influence on the amount of trophoblast passing to the lungs: (1) the nature of the illness causing death (there has long been thought, for example, to be some type of association between circulating trophoblast and eclampsia); (2) the gestational age of the pregnancy (the greater invasiveness of trophoblast in the earlier months might cause greater amounts to enter uterine veins than does in the later months); (3) the degree of uterine contraction (to take extreme cases, more trophoblast might be expected to reach the lungs after a prolonged obstructed labor than after a precipitate delivery); and (4) the length of time elapsing between separation of the placenta and death (trophoblast might survive for no more than a few days within the lung).

For the purposes of this analysis the patients were divided into appropriate groups: for example, (1) those dying after (a) absent or minimal uterine contraction, (b) excessive uterine contraction, or (c) normal uterine contraction; and (2) those dying (d) before separation of the placenta, (e) within 24 hours of separation, (f) later than 24 hours after separation; and in similar further groups for the types of illness and gestational age of the pregnancy. The main conclusion derived from the analysis was, briefly, that the most important factor causing trophoblast to pass to the lungs was the act of separation of the placenta, or "placental commotion." Excessive degrees of uterine contraction have a similar, though less marked, embolism-promoting effect; any promoting influence in this direction that eclampsia may have is due almost certainly to the systemic convulsions and probably increased degrees of uterine contraction that accompany them.

Two further points emerged:

(1) Trophoblast was seen in the lungs of 29 of 39 patients dying during the 24 hours after separation of the placenta, but in only 3 of 13 who died before separation of the placenta. This suggests that we may be wrong in believing that particles of trophoblast pass continually to the lungs throughout the whole of a normal pregnancy. That which we see in the lungs of patients dying after delivery is almost certainly not a true sample of what obtains during pregnancy up to the time of onset of labor.

(2) In contrast to the finding of trophoblast in the lungs of 29 of 39 patients dying within 24 hours of separation of the placenta, trophoblast was found in the lungs of only 4 of 34 dying at varying times from the fifth day of the puerperium to the thirtieth day. It seemed probable from this evidence that, with only occasional exceptions, the maximum length of survival of a particle of trophoblast within the lung is of the order of only 3 days.

W. A. BARDAWIL: Attempts to quantitate trophoblastic emboli in pregnancy lungs were not made. However, rough estimates of the occurrence of embolic trophoblastic cells varied from abundant to moderate or occasional. It is agreed that the quantitative studies advocated by Park may well be conducive to a better understanding of this phenomenon and its relationship to various clinical states of pregnancy. In eclampsia, for instance, the incidence of syncytial deportation is consistently high. In our series, 13 of 16 cases showed these changes. In such cases, in addition to the "placental commotion," excessive uterine contraction, and systemic convulsions suggested by Park, another factor could be considered, that is, the state of premature aging in the placenta that is usually associated with an increase of syncytial knots (compared with the normal), thus affording the primary material for deportation.

In regard to the two cases reported of deportation of apparently normal chorionic villi, the possibility of an unusually severe "placental commotion" must be considered most strongly.

The phenomenon of trophoblastic deportation, in our experience, may occur throughout pregnancy and before separation of the placenta. This was the case in 9 of 11 immature and 2 of 3 term undelivered terminating fatally by reasons unrelated to pregnancy. It is granted that the death struggle may, in part, play a role in the occurrence of this phenomenon.

JOHN BODMAN (*St. Xavier Hospital, London, England*): Some of the problems regarding immune response to trophoblast may hinge upon the presence or absence of a marker protein, a substance that enables other cells to recognize it. Such a protein is present in somatic cells, and part of the complex process that regulates growth control of somatic cells depends upon a restraint imposed by recognition or by being surrounded by contiguous fellow cells each bearing a recognition symbol. If a neighboring cell is removed by injury, the next cell moves in and halts only when met by its opposite from the other side. Were it not for some such mechanism we should have ulcers all over our lives and keloids on our hands.

The implantation of the fertilized ovum creates a wound, an endocrine wound that like most other mucosal injuries, soon becomes suffused with glucose formed by depolymerization of tissue polysaccharides. The blastocyst and its shell of trophoblast are not treated as foreign bodies nor as antigenic substances. Hence, it may be concluded that they lack a marker protein, that they continue to invade, being supported by energy derived from the glucose liberated in the healing process.

While the endometrium has been depolymerizing its polysaccharides to glucose, the placenta has been storing galactogen. At approximately 9 to 10 weeks of gestation, utilization of galactose by fetal tissue begins; for

example, galactose is an essential component of the ABO blood group substances that do not appear until the ninth week.

Recent investigations on fetuin, the earliest known fetal protein, show it to be an acid glycoprotein, comprising as much as 45 per cent of calf serum proteins as far as midterm and falling to levels of approximately 20 per cent at birth. Fetuin has been found in human fetal serum in the first trimester only. Although fetuin is galactose-free in early gestation, a specimen of fetuin prepared from fullterm calf serum was found to contain galactose, and an antiserum to it was prepared from rabbits. The mechanism whereby galactose is incorporated into fetal tissues depends upon the presence of the galacto-waldenase system of enzymes. I suggest, then, that profitable lines of investigation for a study of the problem of an immune response to trophoblast may lie in a study of galactose metabolism by trophoblast and by fetal tissues and in a study of the enzyme systems that catalyze the incorporation of polysaccharides and glycoproteins into trophoblast.

GORDON W. DOUGLAS (*New York University College of Medicine, New York, N. Y.*): The "immunologically oriented reveries" to which W. A. Bardan and B. L. Toy refer are, indeed, one of the most exciting fields for speculation in the realm of biology. The steadily growing interest of surgeons, immunologists, and many other groups in the field of homotransplantation has directed our attention, once again, to the immunological inconsistencies implicit in the fact of intrauterine pregnancy itself. It is clear that we are concerned, not merely with the bizarre behavior of choriocarcinoma, but with the immunological aspects of reproduction.

The importance of deportation of trophoblast, in this respect, lies in the fact that this phenomenon represents direct exposure of fetal tissues to the environment of the maternal host, outside of the special environment of the placental site. The frequency of this phenomenon in pregnancy, as well as its biological significance, has been open to considerable doubt. Thus, its finding in pregnant women dying in convulsions or under conditions of increased intrauterine pressure (toxemia, hydatidiform mole) or intrauterine manipulation (criminal abortion) might raise the suspicion that deportation is largely a terminal and artificial event, rather than a significant process of pregnancy. Furthermore, the demonstration of trophoblast in the lungs of women dying during pregnancy is by no means a routine finding, and requires careful and painstaking search of sections. While clinical manifestations of deportation in the living patient have been reported and undoubtedly do occur, their rarity provides grounds for further doubt.

In this respect, the initial results of an investigation of this phenomenon in the living patient are of interest. At the suggestion of Lewis Thomas of our Department of Medicine, samples of blood were drawn from the various series of uterine veins in the broad ligament in women at the time of cesarean section and before incising the uterus. After appropriate staining (Lewis Thomas, large multinucleated cells morphologically identical to the syncytium of the placenta were found. In 89 samples of blood drawn from the antecubital vein during pregnancy, no cells of this kind were discovered. This eliminates the possibility of the extrusion by these cells into the circulation

manipulation of the uterus, blood was collected from the vena cava of pregnant women through a polyethylene catheter threaded into the femoral vein; cells resembling trophoblast were found in these smears as well. Samples from one patient, at the eighteenth week of pregnancy, revealed an unusually large number of these cells.

The preliminary findings of this study indicate that deportation of the syncytial trophoblast is not an occasional event in pregnancy, but a more or less constant process. Indeed, the bulk of these cells and the number that are found in the blood stream during the course of pregnancy, make it evident that the maternal lung during pregnancy is being showered with trophoblastic material. It is evident, from general clinical experience, that this process evokes no symptoms in the vast majority of pregnant women. In addition, the elimination of these cells from the lung must occur by some mechanism other than the conventional mechanisms of tissue homograft rejection, since tissue sections of the lung from autopsies fail to show the cellular reaction that would accompany this process.

Among the very few observations that form the basis for this field of speculation, mention should be made of the specimens of previllous ova that have been studied at the Carnegie Institution of Washington, Baltimore, Md. The tissues around these early ova show little or no evidence of a process that might be termed rejection. It is not until the third week of pregnancy, when decidua has been formed and villi are present, that the placental site assumes the appearance of a "no man's land." Thereafter the junctional zone between fetal and maternal tissues suggests, from its morphologic appearance, an immunological truce, with minor and continuing depredations on both sides. It is therefore conceivable that failure to achieve this precarious situation in very early pregnancy occurs more commonly than we think, and that the rejection of early ova is not detected by virtue of the fact that their first recognition as foreign tissue occurs at about the time of an expected menstrual period.

CHEMOTHERAPY OF CHORIOCARCINOMA AND RELATED TROPHOBLASTIC TUMORS IN WOMEN

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Our initial experience in the treatment of choriocarcinoma and related trophoblastic tumors with the folic acid antagonist 4-amino-N¹⁰-methylpteroylglutamic acid (methotrexate) has been reported previously.^{1, 2} We shall now describe additional findings in 27 women whose therapeutic response to methotrexate has been observed over a period of two and one-half years.

Our rationale in applying antifolic acid therapy in cases of malignant trophoblastic disease of women stems from earlier laboratory and clinical studies. The tissues of the female genital tract have a high requirement for folic acid that became apparent from experimental studies showing that in the rat or monkey deficient in folic acid, the uterus is incapable of growing in response to administered estrogens.⁴ Moreover, Nelson and Evans³ demonstrated the high fetal requirement for folic acid in the rat. Indeed, Thiersch⁵ reported the induction of therapeutic abortion in women by the administration of aminopterin sodium, the folic acid antagonist. In addition, the clinical phenomenon of the macrocytic anemia of pregnancy responding as it does to folic acid therapy, also appeared to reflect an especially high requirement for folic acid in the rapidly growing fetal and maternal tissues of pregnant women.⁶ Accordingly, we postulated that choriocarcinoma and related trophoblastic tumors that originated in the fetus, chorion and initially involved the uterus might respond to treatment with folic acid antagonists.

Materials and Methods

Patients were admitted to this study only when they presented unequivocal radiological, hormonal, or clinical symptoms of metastatic trophoblastic disease. This collective term refers to those patients in whom prior histological evidence of trophoblastic abnormality had been obtained and in whom subsequent clinical, hormonal, or radiological evidence of disseminated disease was discovered. The criteria for the establishment of the diagnosis are presented for each case in TABLE 1. Therefore, these patients are listed as having either choriocarcinoma or a related trophoblastic tumor.

The 27 cases of trophoblastic disease were classified as follows: choriocarcinoma, 19; chorioadenoma, 3; probable choriocarcinoma, 2; hydatidiform mole and necrotic uterine mass, 1; trophoblastic nodule of the cervix, 1; and inconclusive, 1. Therapy brought about remission with no evidence of residual disease in 5; remission with persistent disease in 11; initial remission and subsequent death in 6; and no initial response in 5. Four of the five patients in whom there was no initial response had undergone only one

course of therapy. The pathological criteria applied are essentially those described by Novak and Seah.⁷ Several observers were asked to review the problem cases and a diagnostic consensus was obtained.

The term hydatidiform mole is used when the intrauterine specimen is composed entirely of molar villi with or without associated blood clot, endometrial tissue, or decidua. The term chorioadenoma indicates that molar villi with associated trophoblast are to be seen within the myometrium or parametrium. The term choriocarcinoma is applied only to lesions characterized by extensive trophoblastic overgrowth with direct invasion of myometrium or parametrium or metastatic spread to extragenital sites. If the histological material suggests choriocarcinoma, but is insufficient to meet the above criteria, the diagnosis is listed as probable choriocarcinoma. Accordingly, nodules of trophoblastic tissue recovered from the vagina or the cervix are not regarded as furnishing a sufficient basis for an unequivocal histological diagnosis of choriocarcinoma. It should be emphasized, however, that, despite such histopathological criteria, all the patients considered here gave clear clinical or hormonal evidence of disseminated disease at the time therapy was instituted.

These tumors invariably provide a unique quantitative biological indicator of their extent and progression in terms of the patient's urinary excretion of chorionic gonadotropic hormone.⁸ We have relied upon the hormonal titer as a major guide in clinical evaluation and therapy. These values are expressed as mouse uterine units excreted over a 24-hour period. The determinations were made at least once weekly by a modification of the method of Klinefelter and his associates.⁹ The urine specimens were collected quantitatively and were refrigerated immediately. In our laboratory, a urinary value of 200 mouse units per 24 hours or less is regarded as normal for previously oöphorectomized women. In those patients in whom such normal urinary titers had been obtained, an additional check for absence of chorionic gonadotropic hormone was made in the serum by a modification of the method of Delfs.¹⁰

In addition, because of the great incidence of pulmonary metastases, we have employed frequent X-ray examinations in the therapeutic evaluation. Furthermore, we carefully followed pelvic manifestations of disease, including uterine enlargement, vaginal implants, uterine bleeding, and secondary obstructive effects on the urinary tract. Finally, central nervous system metastases provided readily assessable neurological criteria of involvement and response to therapy.

All patients were treated with methotrexate according to a specially devised, intensive regimen. The drug was given in 5-day courses at a daily dose of 10 to 30 mg., usually administered intramuscularly and, in some instances, by continuous intravenous drip. The patient's toxicity, especially in terms of stomatitis, intestinal irritation, and cutaneous reactions was constantly appraised. The fluid intake and output were determined daily. Adequate fluid balance was maintained by semiliquid or liquid diets or with parenteral fluids. Stomatitis was treated by frequent oral lavage with diluted viscous lidocaine (Xylocaine) hydrochloride.

TABLE
DATA ON TWENTY-SEVEN PATIENTS

Patient	Clinical status on admission			Histopathology	
	X rays	Pelvic examination	Other findings	Specimens available	Pathological diagnosis
G. B. 26 yr. (1)	Extensive pulmonary metastases	11-cm. suprapubic mass	Debility; anemia from massive uterine hemorrhage necessitating hysterectomy	D & C; uterus	Choriocarcinoma
M. L. 25 yr. (2)	Pulmonary metastases progressing	Posthysterectomy	Pleuritic pain with right hemothorax	Uterus	Chorioadenoma destruens
R. B. 25 yr. (3)	Large single pulmonary metastasis	Posthysterectomy	G.I. bleeding; cerebral metastasis evidenced by diplopia; incoordination; abnormal EEG; retinal hemorrhage	Uterus; scalp nodule	Choriocarcinoma with scalp metastasis
M. E. 17 yr. (4)	Extensive progressive pulmonary metastases	Mass protruding from cervix; uterus 5 cm. above symphysis	Severe dyspnea; pleuritic pain; hyperpyrexia; vaginal bleeding	Biopsy of mass from cervix	Trophoblastic nodules
M. S. 21 yr. (5)	Normal chest	Posthysterectomy	Had undergone pituitary stalk-section elsewhere; debilitated	Uterus	Choriocarcinoma
J. C. 25 yr. (6)	Extensive metastatic disease	Uterus enlarged 3X; 4 cm. labial mass	Extreme dyspnea; fever; debility	Biopsy of left labial mass, uterus	Probable choriocarcinoma; necrotic mass in uterus removed after therapy†
A. A. 29 yr. (7)	Extensive metastatic disease; suprapubic soft tissue mass	Uterus enlarged 3X	Extreme dyspnea; fever, proved thyrotoxicosis	D & C; uterus	Hydatid mole; necrotic mass in uterus removed after therapy
N. B. 25 yr. (8)	Normal chest	Vaginal metastasis 2 cm. X 1 cm.; posthysterectomy	Nausea; weight loss	Vaginal implants & D & C	Probable choriocarcinoma
W. F. 22 yr. (9)	Normal chest; prior metastases regressed under HN2 therapy	Posthysterectomy	Asymptomatic; rising gonadotropin titer only evidence of disease	Uterus	Choriocarcinoma
S. W. 27 yr. (10)	Multiple pulmonary metastases	Posthysterectomy	Weight loss; ambulatory	D & C; uterus	Chorioadenoma destruens
S. C. 22 yr. (11)	Normal chest	Granulation tissue in operative field	Asymptomatic	Uterus	Choriocarcinoma(?)
J. E. 22 yr. (12)	Transient infiltrate in lower left lobe; no clear evidence of metastases	Posthysterectomy	Asymptomatic	D & C; uterus	Choriocarcinoma

WITH METASTATIC TROPHOBLASTIC DISEASE

Methotrexate treatment			Duration of remission (months)	Gonadotropin titers			Current clinical status	Classification of therapeutic response
No. of courses	Duration of therapy (days)	Total dose (mg.)		Before treatment	Titer in remission	Latest titer		
7	180	800	26	4,500,000	50	50	Completely rehabilitated	Remission with no evidence of residual disease by X ray, physical examination and titer (5 cases)
5	58	495	29	40,000	50	50	Completely rehabilitated	
4	36	500	11	1,500,000	50	50	Completely rehabilitated	
4	28	500	10	500,000	100	50	Completely rehabilitated; normal menses	
1	5	125	8	50,000	100	100	Completely rehabilitated; maintained on cortisone and triiodothyronine	
15 7	309 160	1875 875	26	200,000	1,000	500	Asymptomatic; persistent gonadotropin titer responding to repeated therapy	Remission with persistent disease manifested only by gonadotropin titer (7 cases)
10 3*	135 39	1150 37.5	16	12,000,000	500	16,000	Asymptomatic; persistent gonadotropin titer responding to repeated therapy; euthyroid	
5 3*	51 42	625 187.5	8	200,000	1,000	1,000	Asymptomatic; persistent low titer; continues on intermittent therapy	
6	75	750	5	400,000	500	2,000	Asymptomatic; continues on intermittent therapy	
3	31	375	7	500,000	500	300	Asymptomatic; marginally elevated titer	
2*	18	112.5	2	2,000	300	300	Asymptomatic; marginally elevated titer	
6	134	800	5	2,000,000	1,000	2,000	Asymptomatic	

Patient	Clinical status on admission			Histopathology	
	X rays	Pelvic examination	Other findings	Specimens available	Pathological diagnosis
P. M. 23 yr. (13)	Poorly defined multiple densities in both lungs; questionably metastatic	Posthysterectomy	Prior laminectomy for metastatic compression of spinal cord; residual neuropathy both lower extremities	D & C; uterus	Choriocarcinoma
B. W. 31 yr. (14)	Diffuse nodular infiltration both lungs	2-cm. bleeding vaginal implant	Vaginal hemorrhage hemoptysis	Uterus	Choriocarcinoma
L. B. 26 yr. (15)	Multiple large pulmonary metastases	Fundus 3 cm. above pubis	Left hemiparesis following temporoparietal resection for metastasis; papilledema; delirium	Cerebral metastasis	Choriocarcinoma
B. P. 25 yr. (16)	Multiple pulmonary metastases	Posthysterectomy	Marked dyspnea	Uterus	Choriocarcinoma
R. B. 39 yr. (17)	Numerous metastases	Hegar's sign; normal fundus	3-cm. metastatic nodule in left breast; prior lobectomy	Metastases from lung, breast, and brain	Choriocarcinoma
M. D. 28 yr. (18)	Extensive pulmonary metastases	Left adnexal mass	Vaginal bleeding; pleuritic pain	D & C; autopsy	Choriocarcinoma
R. T. 36 yr. (19)	Extensive pulmonary metastases	Posthysterectomy	Cerebral metastasis; abnormal EEG	Uterus and autopsy	Choriocarcinoma
D. S. 23 yr. (20)	Extensive pulmonary metastases	Irregular nodular fundus	Blurred vision; headache; papilledema; Babinski's; abnormal EEG	D & C; autopsy	Choriocarcinoma
B. K. 22 yr. (21)	Extensive pulmonary metastases	6-cm. suprapubic mass	Fever; severe sweating	D & C; autopsy	Choriocarcinoma
M. K. 38 yr. (22)	Normal chest; hydronephrosis & hydroureter	Posthysterectomy; hard pelvic mass	Rt. urinary tract obstruction requiring nephrostomy	D & C; uterus; autopsy	Choriadenoma destruens

(Continued)

Methotrexate treatment			Duration of remission (months)	Gonadotropin titers			Current clinical status	Classification of therapeutic response
No. of courses	Duration of therapy (days)	Total dose (mg.)		Before treatment	Titer in remission	Latest titer		
4*	65	250	E	1,100,000	4,000	4,000	Residual neuropathy	Remission with clinically demonstrable disease (4 cases)
8*	125	895	6	2,000,000	1,000	30,000	Residual pulmonary disease by X ray; vaginal implant completely regressed; therapy continues	
5*	90	350	4	1,000,000	50,000	500,000	Initial regression of pulmonary and intracranial disease followed by rapid progression of pulmonary metastases despite continued methotrexate	
7	127	1025	4	6,000,000	20,000	20,000	Residual pulmonary disease; therapy continues	
7	100	775	7	20,000	500	500	Died abruptly from cerebral hemorrhage due to residual tumor; chest X ray negative prior to death	Expired during relapse following initial remission (6 cases)
9	124	875	3	50,000	2,500	30,000,000	Regression of pulmonary and pelvic metastases followed by rapid progression despite HN2 and DON therapy	
6	84	685	3	8,000,000	10,000	500,000	Regression of pulmonary and intracranial metastases followed by progression despite HN2 and DON therapy	
7 1* 3	70 2 25	875 1000 300	4	4,000,000	5,000	1,000,000	Regression of pulmonary and intracranial metastases followed by progression despite intrathecal methotrexate, HN2 and DON therapy	
6	55	825	3	4,000,000	500,000	500,000	Slight regression of pulmonary and pelvic disease followed by rapid progression and thyrotoxicosis; HN2 and DON therapy without effect	
5	90	350	3	250,000	100	100	Death after 5th course, jaundice and small bowel ulceration attributed to methotrexate toxicity. No tumor tissue identified	

TABLE

Patient	Clinical status on admission			Histopathology	
	X rays	Pelvic examination	Other findings	Specimens available	Pathological diagnosis
E. P. 30 yr. (23)	Huge pulmonary metastases	Many large vaginal implants	Metastatic mass in left calf; herpes zoster; severe dyspnea	Uterus; autopsy	Choriocarcinoma
D. P. 47 yr. (24)	Small nodular metastases	Mass in right vaginal fornix; vaginal hemorrhage	Hydronephrosis and hydroureter requiring right nephrostomy	Autopsy	Choriocarcinoma
A. H. 48 yr. (25)	No apparent metastases	Posthysterectomy	Coma, icterus	Autopsy	Choriocarcinoma
M. R. 51 yr. (26)	Extensive pulmonary metastases	Vaginal implants	Marked dyspnea	Autopsy	Choriocarcinoma
H. K. 31 yr. (27)	Small pulmonary metastases	Posthysterectomy	Icterus; papilledema; postcraniotomy	Brain metastasis; autopsy	Choriocarcinoma

DON: 6-diazo-5-oxo-L-norleucine.

* Given intravenously.

† Nodule composed of syncytiotrophoblastic cells and necrotic tissue.

‡ Material suggestive but not conclusively diagnostic of choriocarcinoma.

§ Subsequent review of additional material from hysterectomy failed to show clear-cut invasion by trophoblast; hence

The patient's hepatic and renal status was determined by appropriate laboratory and clinical studies both before and at frequent intervals during methotrexate therapy. Furthermore, the leukocyte, platelet, and reticulocyte counts were determined at one- to three-day intervals. Successive courses of therapy were not undertaken until all prior signs of toxicity had subsided. All surviving patients have been continuously followed up and autopsy was performed on all cases of death.

Results

The initial clinical status of each patient is sketched briefly in TABLE I. All but 5 were gravely ill on admission. Their most immediate clinical manifestations were respiratory distress due to extensive pulmonary disease and protracted vaginal bleeding. Pulmonary metastases were radiologically demonstrable in 22 patients. Cerebral involvement with evidence of increased intracranial pressure and other neurological manifestations were the presenting clinical features in 5 patients; three were completely asymptomatic and lacked clinical or radiological evidence of disease. Nevertheless, they were admitted for therapy because of persistence of an elevated hormone titer after a previous hysterectomy.

The number of 5-day courses given to any patient varied from 1 to 22. The average number of courses given those patients who received more than

(Continued)

Methotrexate treatment			Duration of remission (months)	Gonadotropin titers			Current clinical status	Classification of therapeutic response
No. of courses	Duration of therapy (days)	Total dose (mg.)		Before treatment	Titer in remission	Latest titer		
3	38	315	—	1,000,000	—	1,500,000	Died of progressive pulmonary insufficiency	Expired during initial courses of therapy without response (5 cases)
1	5	125	—	20,000	—	40,000	Died after first course; probably due to MTX toxicity	
1	3	45	—	—	—	—	Died during first course on third hospital day	
1*	5	75	—	500,000	—	200,000	Died on twelfth hospital day with increasing anoxia. Autopsy suggestive of MTX toxicity	
1	4	100	—	500,000	—	500,000	Died on ninth hospital day	

histological diagnosis of choriocarcinoma is not conclusive.

a single course was 6. The average period of time occupied by a given 5-day course was 12 days, and the average total dose of methotrexate was 620 mg.

Complete remission. The responses of these 27 patients assumed several definite patterns (TABLE 1). In 5 patients we have observed a complete remission with no hormonal, radiological, or physical evidence of recurrence for periods presently extending for 8 to 29 months. By histopathological criteria, 3 of the patients in this group had unequivocal choriocarcinoma; 1 had chorioadenoma; and, in 1, the only tissue specimen available was a biopsy of a fungating mass of necrotic tissue containing a few syncytiotrophoblastic cells exuding from the cervix. However, this patient and 3 others in this group of 5 presented pulmonary metastases by X ray on admission, and 4 had undergone hysterectomy. Two of these cases are summarized below.

Case 3. A 25-year-old woman, Para 1, Gravida 2, had undergone hysterectomy 9 months prior to admission; the surgical specimen revealed choriocarcinoma. She remained symptom-free until 3 weeks prior to admission when, because of severe abdominal pain, she underwent resection of a benign cyst of the left ovary. A few days later, a bleeding scalp nodule was resected and proved to be metastatic choriocarcinoma. Two weeks prior to admission the patient developed nausea and vomiting, severe frontal headache,

and blurring of vision. On admission, intracranial metastasis was manifested by diplopia, left strabismus, and peripapillary hemorrhage in the left fundus; there was a distinctly abnormal electroencephalogram compatible with a right parietal or possible posterior fossa lesion. She passed numerous large tarry stools and required multiple transfusions.

Over a 40-day period she was given 4 courses of methotrexate, each consisting of 25 mg. given daily and intramuscularly for 5 days. Varying degrees of oral and hematological toxicity were encountered with each course. The fourth course induced extremely severe stomatitis with extensive buccal ulceration. The gastrointestinal bleeding subsided during

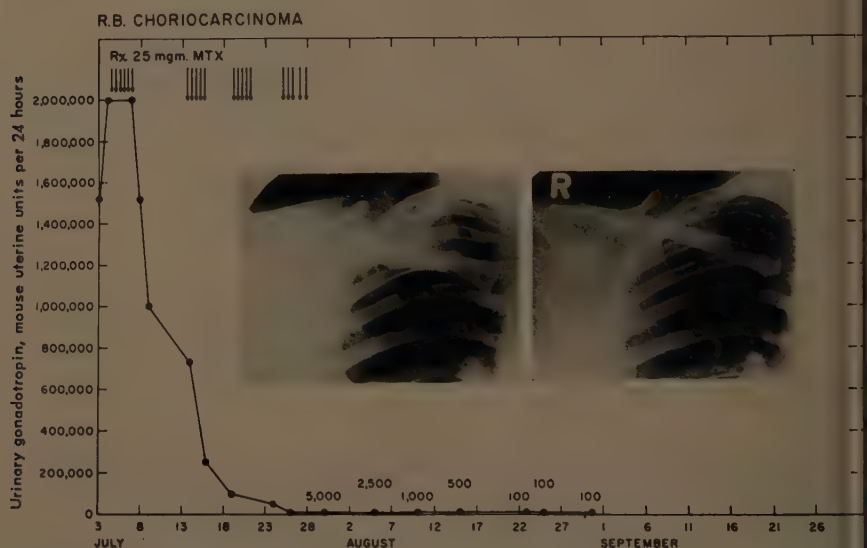


FIGURE 1. Case 3: radiological and hormonal response in patient with choriocarcinoma. Vertical arrows indicate courses of methotrexate.

treatment, and there was immediate reversal of all neurological abnormalities. Electroencephalograms taken before the third course and repeated 20 days later were both normal. As shown in FIGURE 1, the hormone titer fell from 1,500,000 to less than 100 units, and the pulmonary lesion promptly regressed. Since discharge, the patient's chest X rays remain negative; her hormonal titer is within normal limits, and no physical or subjective manifestations of disease are detectable. Her remission has now persisted for 11 months.

Case 4. An 18-year-old woman, Gravida 1, Para 0, had passed a molar pregnancy two and one-half months prior to admission. She then experienced intermittent vaginal bleeding and numerous severe episodes of high fever, which were treated with penicillin and streptomycin. Six weeks prior to her admission, metastatic lung lesions were demonstrated, and results of the Aschheim-Zondek test became strongly positive. The week before admission she was

given numerous blood transfusions and developed severe left lower quadrant pain with recurrent high fever. On the patient's admission, the uterus was enlarged threefold and was extremely tender. A 4-cm. ovoid, red, fungating mass presented from the os cervix. Biopsy showed that this consisted of syncytiotrophoblastic cells associated with considerable hemorrhage and necrosis. She required numerous transfusions and continuous oxygen therapy.

Over the ensuing 53 days she was given 4 courses of methotrexate intramuscularly, each course consisting of 25 mg. daily for 5 days. Little toxicity was encountered after the first course. However, the patient manifested

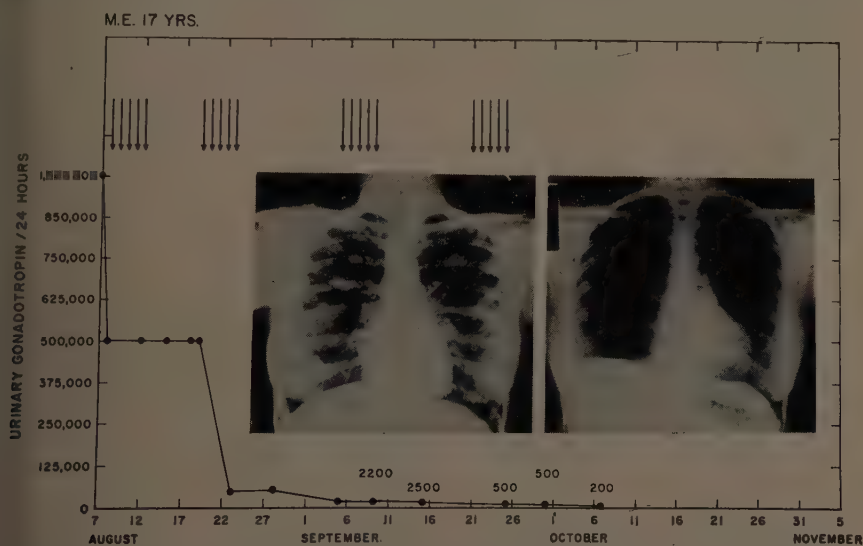


FIGURE 2. Case 4: radiological and hormonal response in patient with trophoblastic nodule. Vertical arrows indicate courses of methotrexate.

severe oral toxicity and marrow depression after the last 3 courses. During the first 2 weeks of treatment she continued to be gravely ill with extreme dyspnea, pleuritic pain, hemoptysis, and lower abdominal pain with tenderness. Ten days later distinct improvement was manifest in terms of radiologically demonstrable regression of the pulmonary metastases (FIGURE 2), marked improvement in respiratory function, visible regression of the cervical mass to a diameter of 2 cm., regression of the uterus to half its previous size, and loss of lower abdominal pain and tenderness. The gonadotropin titer continued to fall to within normal limits (FIGURE 2), and the chest X ray reflected continuing improvement during subsequent courses of therapy. Since discharge, she has presented no hormonal, radiological, or clinical manifestation of recurrent disease; her remission has now persisted for the past 10 months. (Throughout this report, duration of remission is calculated from the time of initial favorable response to therapy as manifested by initial reduction in gonadotropin titer or objective regression of metastases.)

Partial remission. Seven patients have presented a less complete response to therapy; they continue to exhibit unequivocal hormonal evidence of persistent disease. However, each has shown a striking reduction in titer, and 3 have shown complete regression of pulmonary metastases. We can find no radiological or physical evidence of active disease in any of these patients but their hormone titers, although very much lower than before therapy, clearly reflect latent disease. Three of these patients have been treated on successive occasions whenever their titers increased. The hormonal and

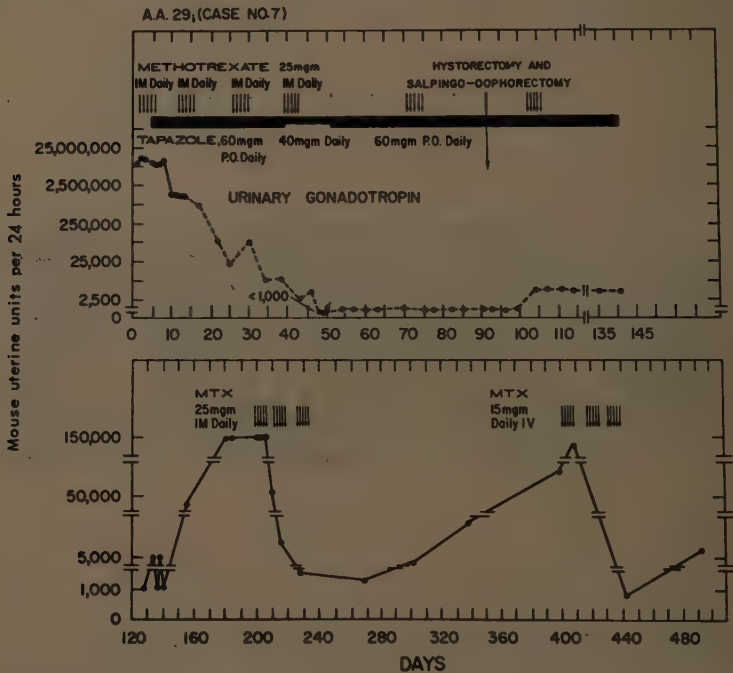


FIGURE 3. Case 7: repeated hormonal response of patient to intermittent methotrexate therapy during 16 months. Vertical arrows indicate courses of methotrexate.

radiological responses of 1 of these patients (Case 7), who has been effectively treated on 3 occasions during the past 16 months, are shown in FIGURES 3 and 4. The X rays of a patient (Case 6) who has been similarly treated during the past 26 months are shown in FIGURE 5.

Transient remission. Ten patients have initially experienced a substantial but transient remission of 3 to 6 months' duration and have subsequently developed apparent refractoriness to methotrexate therapy. All of these patients showed a marked depression of hormone titer during remission. 6 exhibited regression of pulmonary metastases, and 3 showed objective improvement in neurological status. Despite additional methotrexate therapy and, in 4 instances, brief courses of mechlorethamine hydrochloride (nitrogen mustard) and 6-diazo-5-oxo-L-norleucine, all of these patients:

have shown progression of disease, and 6 have died. A marked rise in gonadotropin titer was observed in every patient as the disease became exacerbated (TABLE 2). One patient (Case 16) still has pulmonary disease, but her titer is decreasing as she continues on methotrexate therapy.

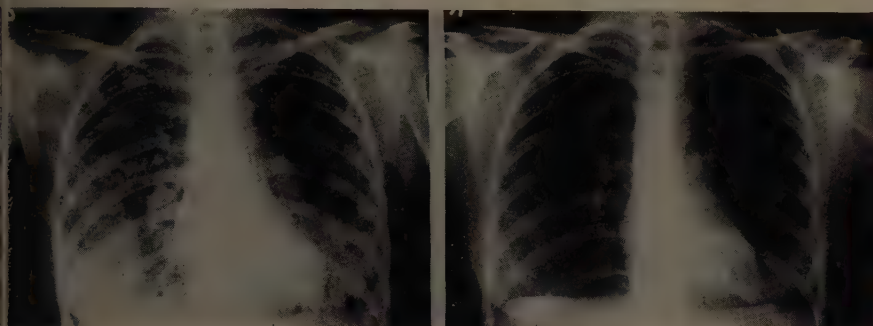


FIGURE 4. Case 7: initial radiological response in patient (left) on admission and (right) after 5 courses of methotrexate in 75 days. Chest X ray 16 months after start of therapy remained negative.

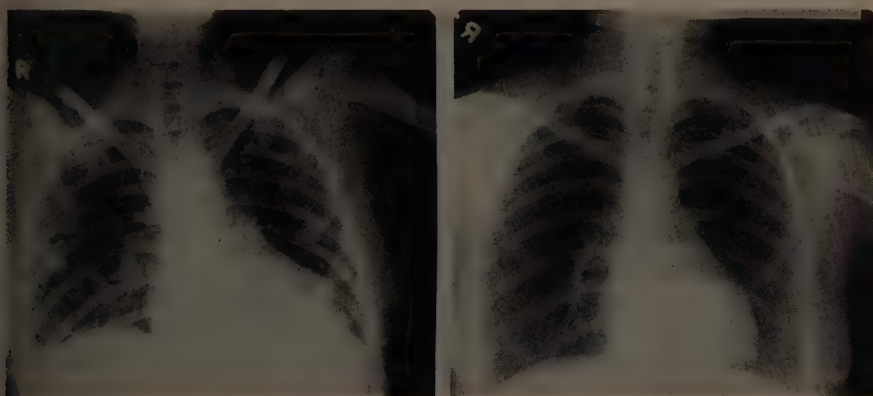


FIGURE 5. Case 6: radiological response in patient (left) on admission and (right) after 6 courses of methotrexate in 125 days. Chest X ray 26 months after start of therapy remained negative.

Among the 6 patients who have died after initial response, 1 (Case 22) died from drug toxicity. This patient had previously shown numerous drug allergies. She had tolerated 4 preceding courses of methotrexate with moderate stomatitis and with evidence of marrow depression, but had exhibited a severe drug rash with each. After the fifth course the patient developed jaundice, enteritis, and a profound leukopenia, and death ensued. Autopsy revealed an acute toxic hepatitis, extensive gastrointestinal ulceration, and extreme marrow destruction. Two other patients who were profoundly debilitated on admission died after an initial course of metho-

trexate. Although the lethal outcome of these two cases was not clearly attributable to drug toxicity, the autopsy findings indicate that this was probably a contributing factor.

These instances of probable lethal toxicity stand in direct contrast to the reversible toxicity encountered during the methotrexate regimen used in these studies. We have now administered 220 individual courses of therapy to these 27 patients and to 13 additional patients with other types of tumors. Consequently, one may conclude that such intensive methotrexate therapy is clinically feasible, although substantial hazard still exists.

Comment

The most frequent manifestations of reversible toxicity are stomatitis, marrow depression, drug rash, anorexia, and nausea with occasional emesis and diarrhea. These manifestations vary widely in intensity from patient to patient and even in the same patient from one course to the next. Accordingly, constant medical surveillance of the patient's status is mandatory, and appropriate supportive medical and nursing care should be readily available. Since methotrexate is excreted largely in the urine, the maintenance of adequate fluid intake and output is of special importance. Preexisting hepatic or renal dysfunction should serve as contraindication to such intensive therapy. Furthermore, this regimen should not be applied in the presence of residual marrow suppression after radiation or other forms of chemotherapy. Since the maximum clinical toxicity is encountered most frequently from 4 to 7 days after the completion of a 5-day course, it is generally not possible to control these manifestations by drug withdrawal. Moreover, it is imperative to delay further therapy until the signs of prior toxicity have subsided.

In the section on *Materials and Methods*, the therapeutic results to date are summarized. If we exclude the 4 patients who received only 1 course of therapy just prior to death, it will be noted that 22 of the remaining 22 showed an initial substantial response to therapy, evidenced primarily in terms of suppression of hormone titer. These responses are summarized graphically in FIGURE 6 and are detailed in TABLE 1. This reflects an almost uniform initial sensitivity of these trophoblastic tumors to methotrexate and indicates the therapeutic potential of this regimen.

It is, however, much more difficult to assess the immediate relationship of the methotrexate therapy to the total clinical course, particularly in those patients who thus far exhibit no residual evidence of disease. Subsidence of even metastatic trophoblastic disease without specific therapy is a well-documented phenomenon.¹¹ Moreover, a clinically benign course has been noted in patients presenting unequivocal metastatic choriocarcinoma.¹¹ On the other hand, the experience reported here and that of other investigators includes fatal cases associated with lesions sometimes regarded as not unquestionably malignant.¹¹

Because of the limited number of cases in which both clinical course and complete histological analysis are available, we have little statistical basis on which to differentiate the outcome in our cases from potentially spontaneous

clinical events. However, the spontaneous occurrence of 5 complete remissions among 27 unselected cases of advanced metastatic trophoblastic disease may be regarded as highly improbable, particularly since 3 of these 5 patients had unquestioned choriocarcinoma. Moreover, the unequivocal

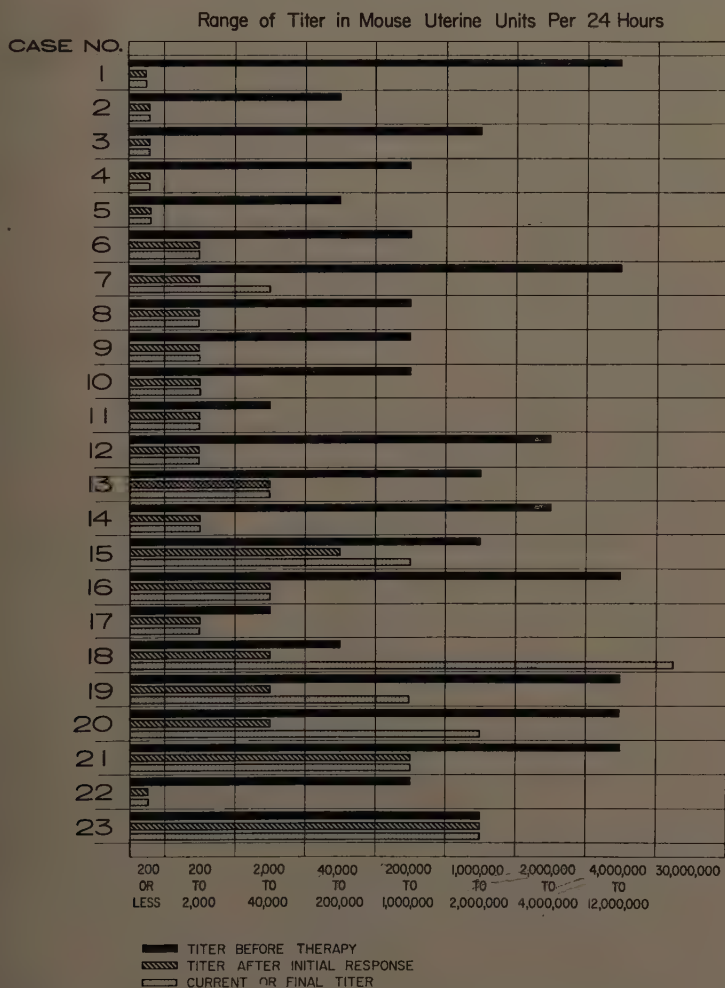


FIGURE 6. Gonadotropin responses in 23 patients who received more than one course of methotrexate.

quantitative evidence of the extreme initial responsiveness of these tumors to methotrexate serves to support the interpretation of a cause and effect relationship between the benign course in these cases and the therapy applied.

The problem of initial and acquired drug resistance is common to all forms of chemotherapy. In the present experience we have noted only one

instance (Case 23) of apparent initial drug resistance. Before she received methotrexate, this patient had had extensive radiation and several courses of nitrogen mustard therapy; this prior treatment may have been a factor in the failure of response to methotrexate.

Since additional intensive methotrexate therapy has usually proved totally ineffective, the acquired methotrexate resistance among previously responsive patients has assumed a fixed pattern in most instances. However, in 3 patients (Cases 6, 7, and 8) some recovery of responsiveness has been noted after varied intervals off therapy (FIGURE 3). For this reason intermittent therapy aimed at maximally obtainable suppression of gonadotropin titer and therefore presumably of trophoblastic disease may ultimately provide the most prolonged periods of remission. In any case, it is too early to establish an optimum mode of management for these patients. For this reason our further trials must remain largely exploratory.

Other forms of chemotherapy for malignant trophoblastic disease have been described. Thus nitrogen mustard has been reported¹² to induce remissions associated with regression of pulmonary metastases and suppression of gonadotropin titer. Also, Li and Magill¹³ have presented data indicating significant response to 6-diazo-5-oxo-L-norleucine. One of our patients (Case 9) had been treated with nitrogen mustard with unequivocal regression of pulmonary metastases 1 year before admission to our service. Accordingly, we have attempted mustard therapy and only brief courses of therapy with 6-diazo-5-oxo-L-norleucine in 4 of our methotrexate-resistant patients with advancing disease. In this advanced clinical phase we observed no effect in any of these patients with either form of therapy. These limited observations suggest a degree of crossover in tumor resistance to the several forms of chemotherapy applied.

Summary

Since fetal and maternal tissues require high amounts of folic acid for their normal metabolism, the therapeutic potential of the folic acid antagonist, 4-amino-*N*¹⁰-methyl pteroylglutamic acid (methotrexate) in the treatment of choriocarcinoma and related trophoblastic tumors has been studied in 27 women. These patients were treated according to a highly intensive regimen, in which certain toxic hazards were present. Therapeutic indexes included (1) serial X rays, (2) physical findings reflecting pelvic, cerebral, and pulmonary involvement, and (3) frequent quantitative determination of the urinary excretion of chorionic gonadotropic hormone.

Remissions varying from 29 to 2 months have been induced in all but 1 patient who had received more than one course of therapy. In 5 patients these continuing remissions are attended by no radiological, physical, or hormonal evidence of residual disease. In 11 patients showing an initial remission, methotrexate resistance has been encountered with varying manifestations of persistent or progressive disease. Six patients responded initially, but subsequently developed resistance to methotrexate and died. One patient died of drug toxicity during remission; drug toxicity may have contributed to the death of 2 other severely debilitated patients.

These experiences demonstrate that metastatic trophoblastic disease is susceptible to treatment and that significant clinical remissions may be achieved. Hence, although the chemotherapeutic regimen is somewhat hazardous, the morbidity and mortality may be regarded as acceptable.

References

1. LI, M. C., R. HERTZ & D. B. SPENCER. 1956. Effect of methotrexate upon choriocarcinoma and chorioadenoma. *Proc. Soc. Exptl. Biol. Med.* **93**: 361-366.
- 1a. LI, M. C., R. HERTZ & D. M. BERGENSTAL. 1956. Therapy of choriocarcinoma and related trophoblastic tumors with folic acid and purine antagonists. *New Engl. J. Med.* **259**: 66-74.
2. HERTZ, R., D. M. BERGENSTAL, M. B. LIPSETT, E. B. PRICE & T. F. HILBISH. 1958. Chemotherapy of choriocarcinoma and related trophoblastic tumors in women. *J. Am. Med. Assoc.* **168**: 845.
- 2a. HERTZ, R. 1958. Dietary impairment of estrogen response in immature monkey. *Proc. Soc. Exptl. Biol. Med.* **67**: 113-115.
3. NELSON, M. M. & H. M. EVANS. 1949. Pteroylglutamic acid and reproduction in rat. *J. Nutrition.* **38**: 11-24.
4. HERTZ, R. & W. W. TULLNER. 1949. Quantitative interference with estrogen-induced tissue growth by folic acid antagonists. *Endocrinology.* **44**: 278-282.
5. THIERSCH, J. B. 1952. Therapeutic abortions with folic acid antagonist, 4-aminopteroylglutamic acid (4-amino P.G.A.) administered by oral route. *Am. J. Obstet. Gynecol.* **63**: 1298-1304.
6. HERTZ, R. 1948. Role of factors of B-complex in estrogen metabolism. *Recent Prog. in Hormone Research. Proc. 1947-1948, Laurentian Hormone Conf.* **2**. Academic Press. New York, N. Y.
7. NOVAK, E. & C. S. SEAH. Choriocarcinoma of uterus. Study of 74 cases from Mathieu Memorial Chorionepithelioma Registry. *Am. J. Obstet. Gynecol.* **67**: 933-961.
8. ZONDEK, B. 1930. Versuch eine biologischen (hormonalen) Diagnostik beim malignen Hodentumor. *Chirurg.* **2**: 1072-1080.
9. KLINEFELTER, H. F., JR., F. ALBRIGHT & G. C. GRISWOLD. 1943. Experience with quantitative test for normal or decreased amounts of follicle-stimulating hormone in urine in endocrinological diagnosis. *J. Clin. Endocrinol.* **3**: 529-544.
10. DELFS, E. 1957. Quantitative chorionic gonadotropin: prognostic value in hydatidiform mole and chorionepithelioma. *Obstet. & Gynecol.* **9**: 1-24.
11. PARK, W. W. & LEES, J. C. 1950. Choriocarcinoma: General review, with analysis of 516 cases. *A. M. A. Arch. Pathol.* **49**: 73-104; 205-241; 361.
- 11a. CASPER, J. 1954. Pathology of chorionic malignancy. *Harefuah.* **46**: 54-56.⁶
12. ANDERSON, H. E., J. D. BISGARD & A. M. GREENE. 1954. Metastatic chorionepithelioma of lungs with nitrogen mustard therapy: Preliminary Report. *A. M. A. Arch. Surg.* **68**: 829-837.
- 12a. ISHIZUKA, N. 1956. Chemotherapy for chorionepithelioma. *Gann.* **47**: 460-462.
13. LI, M. C. & G. B. MAGILL. 1958. Effect of folic acid and glutamine antagonists on chorionic gonadotropin producing tumors. *Abstr. Program of Endocrine Soc.* **No. 53**: 42.

Discussion of the Paper

GORDON W. DOUGLAS (*New York University College of Medicine, New York, N. Y.*): The use of folic acid antagonists, in which R. Hertz and his co-workers have been involved both in laboratory and in clinical investigation, is one of the most promising developments in the field of malignant disease. Whereas the outcome of the final figures is still in the future, there can be no doubt that this group of drugs has a powerful effect on trophoblastic tumors.

My interest in this subject was stimulated by the report of Thiersch¹, who administered aminopterin to 12 patients for whom therapeutic abortion was indicated. Thiersch was preoccupied by the effects of this drug on fetal tissues, but a careful reading of the paper revealed that the A-Z test became negative within 48 hours of completion of treatment in 10 of the 12 cases. In the belief that this could mean only a powerful destructive effect on the placenta as well as the fetus, I conducted a similar experiment in 3 patients prior to therapeutic abortion, between the tenth and fourteenth weeks of pregnancy. Microscopic examination of placental tissues revealed them to be normal in each instance. Aminopterin was then administered to 2 patients with hydatidiform mole between the passage of tissue, and daily determinations of gonadotropin in the serum were carried out. The results of these serum studies are comparable to those presented by Hertz in cases of choriocarcinoma, but rapid expulsion of mole tissue during or immediately following treatment rendered results difficult to interpret. Histological examination of these tissues, again revealed no convincing evidence of drug effect on trophoblast.

I have had the opportunity to observe the effects of methotrexate on choriocarcinoma in 3 patients during the last 2 years. The first patient, with a choriocarcinoma following hydatidiform mole, had multiple chest metastases, vaginal nodules, and a vulvar metastasis. Hysterectomy was followed by methotrexate therapy, given orally according to the schedule described by Hertz. During 3 months, after 3 courses of treatment, all metastatic lesions disappeared and tests for gonadotropin in the serum became negative. The patient remained well until 9 months after operation, when she returned with evidence of a brain metastasis. Despite repeated further treatment with methotrexate, her health followed a downhill course and she expired 22 months after her original diagnosis. Autopsy revealed numerous metastases.

A second patient with metastatic choriocarcinoma in the lungs and scalp was treated with 2 courses of methotrexate. She died during treatment, with evidence of rapidly spreading disease. A third patient with choriocarcinoma of the uterus and vagina 4 months after a term delivery was subjected to hysterectomy. Immediately postoperatively, methotrexate therapy was begun, and resulted in disappearance of the vaginal lesion, with reversal of the biological test for gonadotropin to negative. She remains well after 2 years.

My experience, therefore, is in accord with that of Hertz in finding that response to methotrexate is variable and that suppression of the tumor is by no means indicative of ultimate cure.

Recently I reviewed material on 24 cases of choriocarcinoma, in which pathological data and clinical study were adequate. These included 5 cases from the files of Bellevue Hospital, New York, N. Y. and 19 cases studied from neighboring institutions. Seventeen patients, all but 2 of whom had metastatic disease when first seen, have died. The longest survival among fatal cases was 10 months, except for the patient treated with methotrexate referred to above. However, 7 patients are living after 1 to 19 years and this includes one patient with chest metastases, treated by hysterectomy and X-ray therapy to the chest, who has survived 5 years. As a group, the

surviving cases were noteworthy in that (1) all were treated by hysterectomy and (2) 4 of the 7 had no evidence of metastatic disease. The survival in this small collection of cases, therefore, was 29 per cent after 1 year.

The figures presented by Hertz indicate that of 19 patients with choriocarcinoma who were treated with methotrexate, 5 died during initial treatment, 5 died during relapse after initial response, 4 are in remission with residual disease, and 5 are in remission with no clinically demonstrable disease. The 5 patients last mentioned constitute 26.5 per cent of the group. It would be unfair to compare this figure with that quoted above, since the cases treated by Hertz were, by definition, cases with metastatic disease and fully comparable only to the fatal group in our collection.

There is, therefore, little reason for glowing optimism in the individual case, despite the dramatic effects of methotrexate in many instances. Certainly there is no ground for regarding the folic acid antagonists as an alternative to hysterectomy, even in the young individual, when the diagnosis of choriocarcinoma has been made. The disease remains a highly fatal one, deserving of every means of treatment at our command.

The same cannot be said of chorioadenoma destruens, of which two examples are included in this paper. Since this diagnosis is made, by definition, from hysterectomy specimens, it is necessary to reconstruct the clinical situation in which folic acid antagonists might be considered a very useful alternate to hysterectomy. The young woman who, following expulsion of a hydatidiform mole and curettage, continues to show a subinvolved uterus, positive biological test, and persistent bleeding without evidence of metastatic disease, presents a difficult clinical problem.

Chorioadenoma destruens may be suspected and, if a further curettage fails to reveal tissue in the cavity of the uterus, these suspicions are sharply increased. In this situation, which occurs in perhaps 10 per cent of cases of hydatidiform mole, the use of folic acid antagonists forms a useful alternative to hysterectomy, with the reward of conservation of the uterus and child-bearing function.

I have treated one such patient in these circumstances with aminopterin. There was dramatic relief of symptoms, return of the uterus to normal size, and reversal of the A-Z test to negative. This patient has since delivered a normal term infant, the first, to my knowledge, following folic acid antagonist therapy for this condition.

The use of folic acid antagonists in all patients with hydatidiform moles, as a prophylactic measure against the subsequent development of choriocarcinoma, must be condemned. As Hertz points out, these drugs are profoundly toxic, and the risk that their use entails is hardly justified by the frequency of choriocarcinoma following an uncomplicated hydatidiform mole, even if this type of prophylactic treatment carried a guarantee of certain success.

Reference

- THIERSCH, J. B. 1952. Therapeutic abortions with a folic acid antagonist, 4-aminopteroylglutamic acid (4-amino P. E. A.) administered by the oral route. *Am. J. Obstet. & Gynec.* **63**: 1298-1304.

M. C. LI (*Sloan-Kettering Institute for Cancer Research, Memorial Hospital, James Ewing Hospital, and Kings County Hospital, New York, N. Y.*): I congratulate R. Hertz, my former and senior colleague, on his excellent demonstration and analysis of the effects of the antifolic acid drug, methotrexate upon metastatic choriocarcinoma and related trophoblastic tumors in 2 women. During the last 13 months, since returning to the city of New York, I have been fortunate in having seen or treated 9 women with metastatic choriocarcinoma, 3 with chorioadenoma destruens, and 2 with trophoblastic tumor without definitive classification. Of the 14 cases, only 7 patients have been treated adequately and hence are valid for therapeutic evaluation. The experiences and results are summarized in TABLE 1.

TABLE 1

Case No. and hospital	Diagnosis and extent of lesion	Initial urinary chorionic gonadotropin titer (I.U.)	Therapy	Results	Duration of remission
1 Long Island College, Brooklyn, N. Y.	Choriocarcinoma of uterus and metastasis to vagina	50,000 to 120,000	Methotrexate	CGH negative; resolution of vaginal mass	5 months
2 New York, N. Y.	Chorioadenoma destruens of uterus and metastasis to lungs	200,000	Methotrexate	CGH negative; regression of pulmonary lesions	2 months
3 Memorial Center, New York, N. Y.	Chorioadenoma destruens mass in vaginal stump	600,000 to 800,000	DON	CGH negative; resolution of vaginal mass	10 months
4 Lenox Hill, New York, N. Y.	Choriocarcinoma metastasis to lungs	200,000	Methotrexate	CGH 5,000 I.U.; substantial regression of lung lesions	3 months
5 Memorial Center	Choriocarcinoma metastasis to lungs, kidney, brain	200,000	Methotrexate	CGH reduced to 10,000 I.U.; no response of lung lesions	6 weeks
6 Memorial Center	Choriocarcinoma to lungs and kidney	600,000	DON	No response	0
7 Kings County, New York, N. Y.	Chorioadenoma destruens of uterus	800,000 to 1,200,000	DON	CGH reduced to 5,000 I.U.	3 months

The methotrexate therapeutic regimen used is essentially the same as that recommended originally by Li *et al.*^{1, 2} except in Case 5, in that a daily maintenance dose of 10 mg. *per os* was given over a 6 weeks' period. The drug was interrupted temporarily twice, however, because of evidence of toxicity. The failure to show any substantial clinical improvement probably was attributable to the manner in which the drug was given. The term total remission

is applied only to those who have shown persistent negative values of urinary chorionic gonadotropin by the rat ovarian hyperemia test and complete disappearance of clinical evidence of disease. Thus the pattern of response from these patients is well in agreement with that just reported by Hertz. The effect of methotrexate upon urinary chorionic gonadotropin excretion in Case 1 is illustrated by FIGURE 1.

V.O. 26 YRS. ♀ CHORIOCARCINOMA

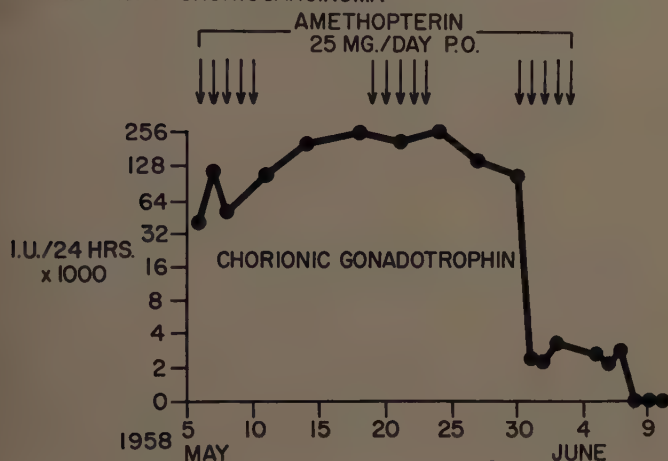


FIGURE 1. The response of urinary chorionic gonadotropin to methotrexate therapy in Case 1.

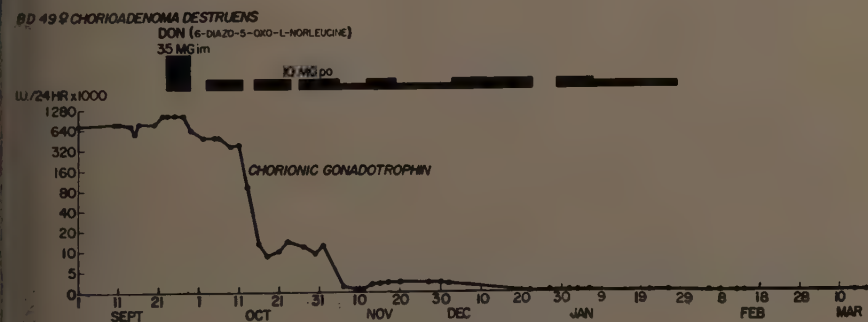


FIGURE 2. The response of urinary chorionic gonadotropin to DON therapy in Case 3.

The glutamine antagonist 6-diazo-5-oxo-L-norleucine (DON) has been reported to be effective in causing fetal death or abnormality in animals by Dagg *et al.*³ and by Thiersch.⁴ It had been used in the treatment of other metastatic cancers.⁵ We have treated three patients with the drug on a daily maintenance basis. The dosage used and response of Case 3 is illustrated by FIGURE 2. As shown in TABLE 1, one patient with metastatic choriocarcinoma died of her advancing disease in spite of 6 weeks of DON therapy. Thus the therapeutic value of this drug is guarded; however, in cases where potential

toxic hazard from methotrexate therapy is expected, DON therapy may be considered as an alternative, since DON does not rely solely on renal or liver function for excretion or detoxification.

In summary, I share Hertz's conservatism in not making any decisive therapeutic claim because of the variation of the biological behavior of this group of tumors, because of the apparent recurrence of disease after total remission induced by the therapeutic modalities, and because of our short term experiences to date with the antifolic acid compound. Nevertheless from our last three years' experiences in the treatment of this disease, I must

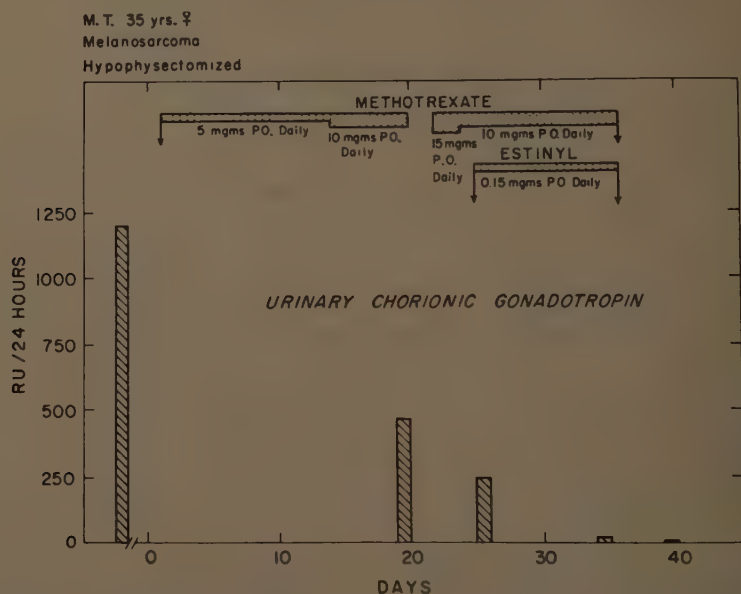


FIGURE 3. The response of urinary chorionic gonadotropin in a woman with malignant melanoma treated with methotrexate.

say that this is a definitely valuable method of therapy at least for the present, in that, undoubtedly, a number of patients who were considered terminal and hopeless before the use of methotrexate have gone back to normal life and well-being. The strikingly high incidence of initial response to the antifolate drug is indeed a rare accomplishment in the field of cancer chemotherapy.

Due to the limitation of time Hertz did not have the opportunity to mention the rationale and background in the development of the methotrexate regimen in the treatment of this group of tumors. While acknowledging Hertz's work in demonstrating the inhibition of estrogen-induced growth of female genital tract in experimental animals by the antifolic compound reported in 1948 and Thiersch's work in inducing fetal death both in animals and women⁸ with aminopterin, the impetus that stimulated the use of methotrexate in the treatment of choriocarcinoma was from the incidental laboratory findings that are illustrated by FIGURE 3.

In 1953, while I was working under O. H. Pearson at the Sloan-Kettering Institute and at the Memorial and James Ewing hospitals, we were interested in obtaining an antiestrogen agent for the treatment of metastatic breast carcinoma. Hertz's work with aminopterin showing inhibition on estrogen-induced growth of chick oviduct stimulated us to apply his findings to a young hypophysectomized woman with metastatic malignant melanoma. The antifolic drug methotrexate was given for about 3 weeks prior to estrogen administration. Thereafter, it was given in combination with ethinylstradiol for another 2 weeks. Daily vaginal smears were made, and the percentage of cornified cells was counted as an index of estrogen activity. There was a rapid rise of percentage of cornification of the vaginal epithelium as soon as the estrogen was started. It remained almost 100 per cent at the end of the study. An incidental finding was that, for some strange reason, there was a substantially elevated chorionic gonadotropin titer in the patient's urine. Pathologic slides from biopsies of her tumors and later from autopsy materials were reviewed on several occasions, but showed no signs of trophoblastic or embryonal elements. It was simply a matter of routine that we did the urinary chorionic gonadotropin assay on a biweekly basis. There was a gradual decline of the titer after starting methotrexate and, when the study was completed, the urinary chorionic gonadotropin titer became negative. It was this incidental laboratory finding that led me to speculate on the possible therapeutic effect on the chorionic gonadotropin-producing tumors.^{1, 2}

The failure of response in choriocarcinoma of testis to antifolic drug therapy is probably on the basis of biological differences. Fetal death or teratogenic abnormalities induced by the administration of the antimetabolites or the alkylating agents can be obtained only in specific stages of the early embryo.⁹ The stages immediately prior to and following are perhaps relatively non-sensitive. Since testicular choriocarcinoma often coexists with embryonal carcinoma and/or teratocarcinoma, this may allow us to speculate that these cells probably represent several different stages of maturity of the embryo that would not respond to chemotherapeutic agents. Another important factor that may account for apparent therapeutic success in certain women is that choriocarcinoma is a transplanted tumor originating from the fetus. The presence of an immune mechanism that may have potentiated the chemotherapeutic effect is possible. Choriocarcinoma of testis is a spontaneous tumor; hence such an immune mechanism would not be expected to develop.

Acknowledgment

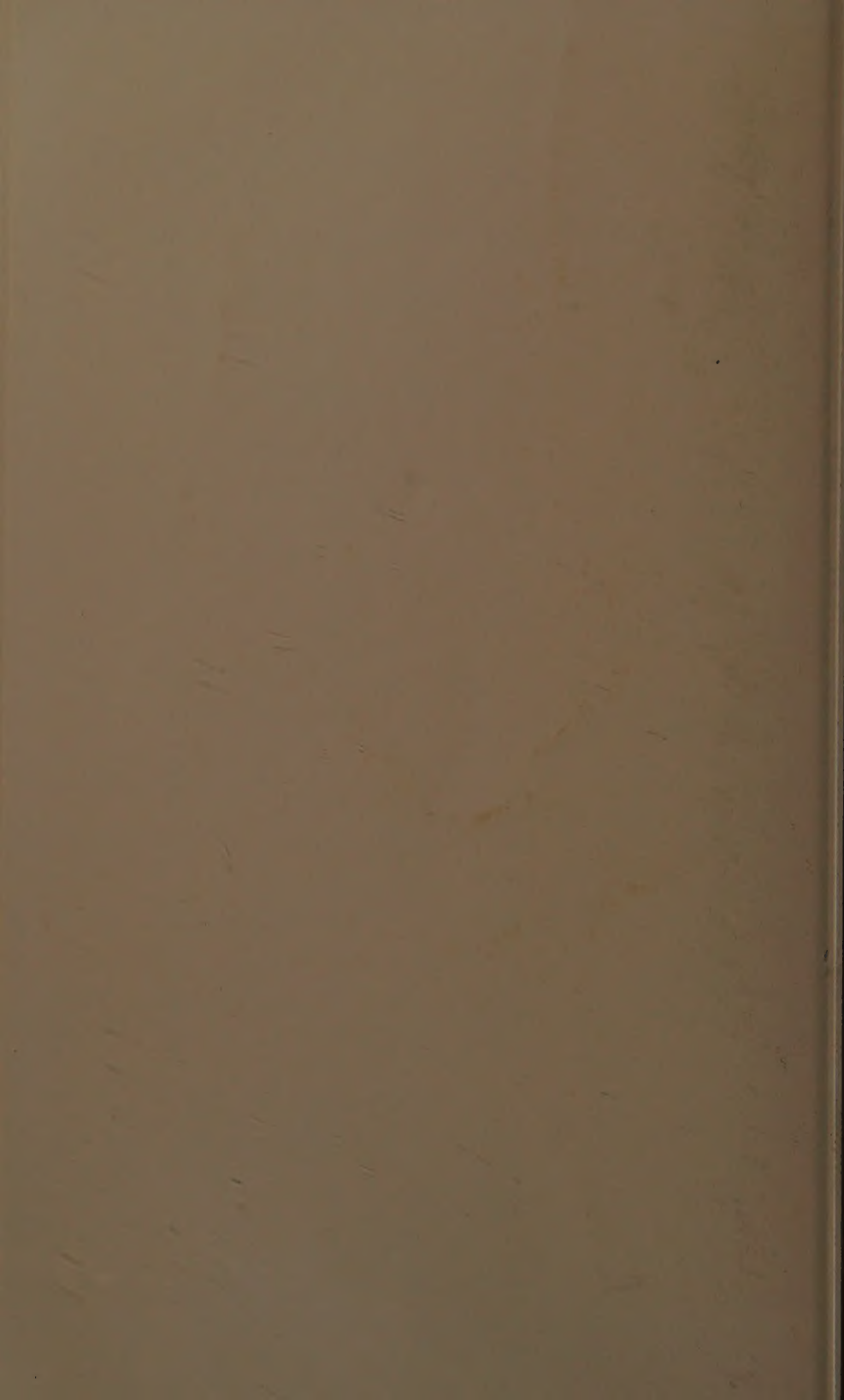
I am grateful to Morris Glass, Hugh Barber, R. Gordon Douglas, and John Masterson for permitting me to study their patients in obtaining the data shown in TABLE 1.

References

1. LI, M. C., R. HERTZ & D. B. SPENCER. 1956. Effect of methotrexate therapy upon choriocarcinoma and chorioadenoma. *Proc. Soc. Exptl. Biol. Med.* **93**: 361.
2. LI, M. C., R. HERTZ & D. M. BERGENSTAL. 1958. Therapy of choriocarcinoma and related trophoblastic tumors with folic acid and purine antagonists. *New Engl. J. Med.* **259**: 66.

3. DAGG, C. P., D. A. KARNOFSKY, C. A. LACON & J. RODDY. 1956. Comparative effects of 6-diazo-5-oxo-L-norleucine and O-diazoacetyl-L-serine on the chick embryo. *Proc. Am. Assoc. Cancer Research.* **2**: 101.
4. THIERSCH, J. B. 1957. Effect of 6-diazo-5-oxo-L-norleucine (DON) on the rat litter *in utero*. *Proc. Soc. Exptl. Biol. Med.* **94**: 33.
5. MCGILL, G. B. *et al.* 1957. Pharmacological and initial therapeutic observations on 6-diazo-5-oxo-L-norleucine (DON) in human neoplastic cancer. *Cancer.* **10**: 1138.
6. HERTZ, R. 1948. Interference with estrogen-induced tissue growth in the chick genital tract by a folic acid antagonist. *Science.* **107**: 300.
7. THIERSCH, J. B. & F. S. PHILIPS. 1950. Effect of 4-amino-pteroylglutamic acid on early pregnancy. *Proc. Soc. Exptl. Biol. Med.* **74**: 204.
8. THIERSCH, J. B. 1952. Therapeutic abortions in man with a folic acid antagonist 4-amino-pteroylglutamic acid administered by the oral route. *Am. J. Obstet. Gynecol.* **63**: 1928.
9. MURPHY, M. L. & D. KARNOFSKY. 1956. Effect of azaserine and other growth inhibiting agents on fetal development of rat. *Cancer.* **9**: 955.





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